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ORIGINAL ARTICLES / ORIGINALNI RADOVI

Dr. Ferdinand Blumenthal (1870–1941), a well-known German oncologist, one of the founders of cancer research in Germany, lived in Belgrade, the Kingdom of Yugoslavia at that time, from 1933 to 1937. During that period he held lectures at the Faculty of Medicine in Belgrade and significantly improved cancer research and treatment in the Kingdom of Yugoslavia (see pp. 847–851).

Dr Ferdinand Blumental (1870–1941), poznati nemački onkolog, jedan od utemeljivača istraživanja kancera u Nemačkoj, živeo je u Beogradu (u to vreme Kraljevina Jugoslavija) od 1933. do 1937. godine. Tokom tog perioda držao je predavanja na Medicinskom fakultetu u Beogradu i značajno je unapredio istraživanja i lečenje karcinoma u Kraljevini Jugoslaviji (vidi str. 847–851).

ORIGINAL ARTICLES

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Anatomical characteristics of the furcation area and root surfaces of multi-rooted teeth: Epidemiological study

Anatomske karakteristike furkacija i korenova višekorenih zuba – epidemiološka studija

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Abstract

Background/Aim. Knowledge of numerous variations in anatomical features of furcation area is a prerequisite for the achievement of more predictable results in the therapy of multi-rooted teeth with furcation involvement (FI). The aim of the study was to evaluate the morphological characteristics of extracted molars of adult population in Belgrade, Serbia. **Methods.** In total, 468 extracted first and second molars, both mandibular and maxillary, were measured. The values of root trunk lengths and root lengths, diameter of furcation entrances (FE), distance between the roots and depth of root concavity were analysed. **Results.** The maxillary first molars had significantly higher root trunk lengths values than the second molars. As for the mandibular molars, FE was smaller than 1 mm. The distance between the roots was more than 2 mm at the third level of measurement. **Conclusion.** The buccal FE of maxillary molars was the lowest. The root concavity of the second mandibular molars was higher from the lingual aspect.

Key words:
furcation defects; molar; odontometry; tooth root.

Apstrakt

Uvod/Cilj. Poznavanje mnogobrojnih varijacija anatomskih karakteristika furkacija (furkacionih regija) je preduslov za postizanje predvidivih rezultata terapije furkacionih defekata višekorenih zuba. Cilj ovog istraživanja je bio procena morfološke karakteristike izvađenih zuba adultne populacije u Beogradu. **Metode.** Merenja su vršena na ukupno 468 izvađenih prvih i drugih molara i gornje i donje vilice. Analizirane su izmerene vrednosti dužine korenskog stabla i dužine korenova, prečnik ulaza u furkaciju, odstojanje između korenova i dubina korenskog konkaviteta. **Rezultati.** Prvi gornji molari su imali signifikantno veće vrednosti dužine korenskog stabla od drugih gornjih molara. Kod donjih molara, prečnik ulaska u furkaciju bio je manji od 1 mm. Na trećem nivou merenja, odstojanje između korenova imalo je vrednost veću od 2 mm. **Zaključak.** Prečnik ulaska u furkaciju sa bukalne strane gornjih molara bio je najmanji. Dubina korenskog konkaviteta drugih donjih molara bila je veća sa lingvalne strane.

Ključne reči:
furkacija, defekti; molari; odontometrija; zub, koren.

Introduction

During periodontitis, the process of periodontal tissue breakdown can affect the alveolar bone loss in furcations of multi-rooted teeth. The American Academy of Periodontology (AAP) has defined furcation as "an anatomical part of a multi-rooted tooth where roots begin forking" and a furcation involvement (FI) tooth is referred to as a tooth with "pathological resorption of the supporting alveolar bone within a furcation" [1].

Consequently, in the course of deepening the gingival sulcus and periodontal pocket formation, the root surface of the tooth becomes exposed, thus increasing the area suitable for the adherence of dental plaque and colonization of periodontopathic bacteria [2]. At the same time, the progression of periodontal lesion destruction depends on root morphology [3].

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Another specific feature of the periodontal pocket in the region of tooth furcation is the existence of its horizontal dimension, toward the interior part of the furcation as well as its vertical dimension along the root due to the bone and attachment loss. There is a clear classification of FI based on the degree of horizontal and/or vertical probe penetration [4].

The molars demonstrate the highest rate of periodontal destruction in untreated disease and suffer the highest frequency of loss for periodontal reasons [5–7]. On the other hand, the teeth with FI respond less favourably to the conventional periodontal therapy unlike the ones with no FI molars or one-rooted teeth [8]. The prognosis and treatment of those teeth can be challenging both for dentists when approaching adequate instrumentation of the affected area and for patients who are involved actively in maintaining the condition of periodontal tissues. However, a large number of treatment methods, including the nonsurgical and surgical mechanical debridement, furcation plasty, tunnelling procedures, hemisections, root resections and regenerative procedures can manage the anatomic area.

Both long-term retrospective studies as well as prospective studies showed less favourable reports for the FI molars. Hirschfeld and Wasserman [9] indicated that, in the period of 22 years, the patients included in a supportive periodontal therapy program lost 7.1% of all teeth for the periodontal reasons. The matching result for the multi-rooted teeth with FI was 31%. Other similar studies confirmed these findings, such as those by McFall [10], Goldman et al. [11]. The findings of the studies done by Loos et al. [12] and Claffey and Egelberg [13] showed that FI molars had a poorer response to non-surgical periodontal therapy and tend to lead to gradual attachment loss.

The practical applications from the AAP Regeneration Workshop defined: "the factors other than systemic, which affect or limit successful treatment, are local and specific to the anatomy of the furcation region, such as root trunk length (RTL), root concavities (RC), root proximity/convergence, furcation entrance (FE) width" [14]. Furthermore, the local factors related to the course of periodontitis of multirooted teeth are the root length (RL), distance between the roots (DBR) and developmental abnormalities (e.g., enamel pearls, cement-enamel projections, accessory endodontic canals and bifurcation ridges) [15, 16].

RTL refers to the distance between the cement-enamel junction and furcation [17]. RTL in addition to the amount of bone loss were suggested to supplement the furcation classification [18]. Moreover, the root trunks can be classified into different types according to Hou and Tsai [19], based on the ratio of root trunk height to RL. The root trunk surface areas of the mandibular and maxillary molars comprise on average 31% and 32% of the total root surface area respectively [20, 21]. Therefore, a root body is compromised by the loss of horizontal attachment, which leads to furcation invasion, the consequence of which is the loss of one third of the total periodontal support of a tooth [22]. Debridement and maintenance of the furcation area are made difficult due to the size of FE. The study of dos Santos et al. [23] shows that the majority of FEs are smaller than the dimensions and curvature of the treatment curette.

Both prognosis and treatment plan are equally influenced by the position of roots of multi-rooted teeth affected by periodontitis[24]. The convergent roots, representing small DRT, are more difficult to regenerate and disease progression in the FI teeth is accelerated. In the case of some specific anatomical characteristics, a multi-rooted tooth implies the presence of the concavity in the furcation area. The role of RC in physiological conditions is to improve the resistance of a tooth to the strong mastication forces [25]. On the other hand, the presence of RC is an important additional local etiological factor supporting the retention of the biofilm.

The aim of the present study is to evaluate the most important anatomic features, such as RTL, RL, diameter of FE, DBR at different levels as well as the depth of RC of maxillary and mandibular molars of adult population in Belgrade, Serbia.

Methods

This epidemiological study included extracted permanent first and second molars: 134 first and 97 second mandibular molars, as well as 121 first and 116 second maxillary molars. The study was conducted in accordance with the Declaration of Helsinki 1975, as revised in 2002. The protocol was approved by the Ethics Research Committee of the Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia. The teeth were collected at the Department of Oral Surgery, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia.

In order to be included in the study, the teeth should have intact crowns and complete roots as well as the preserved cemento-enamel junction, furcation area and the area coronal and apical from the furcation.

After extraction, the teeth were washed with water and immersed in 15% hydrogen peroxide for a period of 24 hours. The debris comprised of the periodontal fibers was removed by a hand curette and the residual supragingival and subgingival calculi were eliminated carefully by an ultrasonic scaler. The measurements were performed by using the electronic caliper (Electronic caliper; Orion 31,170, 210) and a compass with one screw.

The following parameters were measured on the selected molars: RTL corresponded to the area of tooth extending from the cement-enamel junction to the furcation entrance. On maxillary molars, this length was measured at the buccal, mesial and distal sides of the root trunk, and on the mandibular molars, at the buccal and lingual sides. RL represented the distance from the cement-enamel junction to the root apex. RL was measured for the mesial and distal roots of mandibular molars, and for all three roots of maxillary molars. The diameter of the FE was measured between the mesial and distal roots of mandibular molars from the buccal and lingual sides, while on the maxillary molars, it was measured between the mesial and distal roots, the mesial and palatal roots as well as the distal and palatal roots. DBR of each tooth were measured at five levels, from each side of tooth. The first level was located 1 mm apically from the furcation entrance and each subsequent measurement was per-

formed 1 mm apically from the previous point. The last level of measurement was located 5 mm apically from the furcation entrance. The depth of the RC is located coronal from the furcation, on the roof of the furcation and apically from the furcation. Consequently, the concavity depth measurements were performed at three levels. The teeth were cut in the same furcation region followed by 2 mm coronary from the furcation and about 2 mm apically from the furcation. The cutting was done by a high power turbine handpiece (Kavo SUPERTORQUE lux 2 640db) using a fissure diamond drill of 0.12 mm in diameter. After noticing these concavities, they were measured at the deepest parts.

The statistical analysis was performed using the STATGRAPHICS® Centurion XVI. I. The program was designed to compare two samples of data and calculate various statistics and graphs for each sample. The extracted teeth were used as units of analysis. RTL, RL and FE diameter were reported using the parameters of central tendency (mean, median) and variations (standard deviation, min, max), and 99.9% confidence interval (CI). One Way ANOVA was used for evaluating the mean values of the distance between the roots and depth of RC. The statistical significance of differences in the observed parameters between the groups, at each observation point, was analyzed by using the paired samples: *t*-test, F-test, W-test. The Kolmogorov-Smirnov test was used to compare distributions of the two samples. The test was performed by calculating the maximum distance between the cumulative distributions of the two samples. In the Multiple Range Tests, these intervals were used to determine the significant difference of the mean values. The statistical significance of all the tests was defined as $p < 0.001$.

Results

There was a statistically significant difference between the buccal and lingual sides of the RTL in the group of mandibular first molars (Table 1). Unlike the lingual side, the mean values of RTL at the buccal side of the first molar were significantly lower compared to the buccal sides of the second molar. The RTL value for the first maxillary molars was significantly higher compared to the second molars regarding the RTL at the mesial aspect. Both in the first and the second molars, RTL on the distal aspect was significantly bigger compared to the RTL on either mesial or buccal aspect.

The mean value of mesial and distal RL of the second mandibular molars was significantly higher than the value of the first molars (Table 1). The mean value of mesiobuccal and palatal RL-s of the first maxillary molars was significantly higher compared to the second molars, in contrast to distobuccal. For the maxillary first molars, the mean lengths of mesiobuccal and palatal roots (12.36 ± 1.71 mm and 13.09 ± 1.74 mm, respectively) were longer than the distobuccal roots (11.8 ± 1.73 mm). As opposed to that, the mean length of the mesiobuccal root of the maxillary second molar was shorter (11.53 ± 0.11 mm) compared to the mean length of distobuccal and palatal roots (12.50 ± 0.13 mm and 12.66 ± 1.43 mm, respectively).

The results in Table 1 demonstrate that a statistically significant difference was measured between the mean values of the buccal and the lingual FE of the mandibular molars. The buccal FE was wider on the first molars than on the second molars.

Table 1

Root trunk length (RTL), root length (RL) of the first and second mandibular and maxillary molars and furcation entrance of mandibular and maxillary molars

Parameters	Mean ± SD, mm (minimum-maximum)	Median, mm	Cofficient of variation (%)	*p*
Mandibular molars (1st and 2nd)				
RTL				
BRT				
1st	3.27 ± 0.71 (2.26–5.23)	3.03	21.89	0.000
2nd	3.66 ± 0.40 (2.76–4.36)	3.66	10.93	
LRT				
1st	4.30 ± 0.85 (3.01–6.94)	4.12	19.86	0.039
2nd	3.73 ± 0.43 (2.9–4.58)	3.67	11.47	
RL				
M				
1st	12.80 ± 1.68 (9.35–16.34)	12.98	13.15	0.000
2nd	13.62 ± 0.65 (11.25–14.35)	13.81	4.75	
D				
1st	12.83 ± 1.66 (9.54–16.01)	13.01	12.96	0.000
2nd	13.67 ± 1.15 (11.23–23.0)	13.78	8.40	

Table 1 (*continued*)

Parameters	Mean ± SD, mm (minimum-maximum)	Median, mm	Cofficient of variation (%)	*p*
FE				
BFE				
1st	0.77 ± 0.05 (0.65/0.87)	0.78	6.43	0.000
2nd	0.57 ± 0.06 (0.45–0.7)	0.57	10.41	
LFE				
1st	0.67 ± 0.05 (0.54–0.8)	0.68	8.06	0.000
2nd	0.44 ± 0.05 (0.3–0.54)	0.44	11.80	
Maxillary molars (1st and 2nd)				
RTL				
BRT				
1st	4.53 ± 0.85 (3.24–7.21)	4.38	18.72	1.366
2nd	3.71 ± 0.41 (2.87–4.4)	3.67	10.93	
MRT				
1st	4.85 ± 0.87 (3.41–7.57)	4.75	17.90	0.000
2nd	4.57 ± 0.47 (3.74–5.56)	4.49	10.19	
DRT				
1st	3.91 ± 0.70 (2.45–5.80)	3.82	17.88	0.031
2nd	3.83 ± 0.40 (2.90–4.52)	3.80	10.39	
RL				
MB				
1st	12.36 ± 1.71 (9.12–16.5)	12.32	13.87	0.000
2nd	11.53 ± 0.11 (11.29–11.76)	11.54	0.95	
DB				
1st	11.80 ± 1.73 (8.74–16.84)	11.54	14.67	2.087
2nd	12.50 ± 0.13 (12.27–12.77)	12.49	1.06	
PAL				
1st	13.09 ± 1.74 (10.01–17.2)	12.81	13.29	0.000
2nd	12.66 ± 1.43 (9.75–16.86)	12.22	1.94	
FE				
BFE				
1st	0.60 ± 0.08 (0.43–0.79)	0.59	13.82	0.000
2nd	0.53 ± 0.06 (0.42–0.67)	0.53	11.14	
MFE				
1st	1.37 ± 0.08 (1.13–1.52)	1.38	5.67	0.848
2nd	1.37 ± 0.07 (1.21–1.53)	1.36	4.96	
DFE				
1st	1.14 ± 0.04 (1.04–1.25)	1.14	3.79	0.000
2nd	0.9 ± 0.07 (0.75–1.04)	0.9	7.43	

BRT – buccal root trunk; LRT – lingual root trunk; MRT – mesial root trunk; DRT – distal root trunk; M – mesial RL; D – distal RL; MB – mesiobuccal; DB – distobuccal; PAL – palatal; BFE – buccal furcation entrance; LFE – lingual furcation entrance; MFE – mesial furcation entrance; DFE – distal furcation entrance; SD – standard deviation.

The maximum measured value 0.87 ± 0.05 mm was at the buccal side of the first molar and the minimum 0.30 ± 0.05 mm at the lingual FE of the second mandibular molar. For the maxillary molars, FE was wider on the first molars compared to the second molars, except for the mesial FE. This FE was the widest and equal for both molars, approximately 1.37 ± 0.07 mm. The narrowest FE of 0.54 ± 0.05 mm was measured at the buccal side, which was, fortunately, more accessible area for scaling and root planning than the mesial or distal one.

DBR of the first mandibular molars was significantly higher than DBR of the second molars (Table 2). DBR measured at the buccal side 1mm apically from the FE was only 0.26 ± 0.04 mm, but at the fifth level of measurement (5 mm from the FE), it reached the value of 4.03 ± 0.14 mm. Lingual DBR ranged from 0.65 ± 0.06 mm to 3.67 ± 0.07 mm. DBR of maxillary first and second molars had a significant difference only regarding the values of distobuccal and palatal roots. The smallest values were measured between the mesiobuccal and palatal roots of the second molars (from 1.4 ± 0.06 mm to 2.53 ± 0.06 mm, that is, from the first to the fifth level, respectively). The highest values were measured between mesiobuccal and palatal roots of the first molars (Table 2). These values ranged from 2.23 ± 0.08 mm to 6.69 ± 0.013 mm from the first to the fifth level, respectively.

Table 2
The distances between the roots of first and second mandibular and maxillary molars and between maxillary molars

Parameters	Mean ± SD, mm (minimum-maximum)	Median, mm	Cofficient of variation (%)	*p*
Mandibular molars (1st and 2nd)				
Bdm-d				
1d				
1st	0.86 ± 0.04 (0.26–0.87)	0.87	5.11	0.000
2nd	0.68 ± 0.06 (0.57–0.8)	1.54	8.50	
2d				
1st	1.53 ± 0.07 (1.37–1.68)	0.68	4.49	0.000
2nd	1.33 ± 0.05 (1.2–1.44)	1.33	3.70	
3d				
1st	2.19 ± 0.10 (1.89–2.6)	2.18	4.62	0.000
2nd	2.00 ± 0.05 (1.86–2.09)	1.99	2.32	
4d				
1st	2.83 ± 0.13 (2.53–3.25)	2.83	4.45	3.551
2nd	2.65 ± 0.05 (2.51–2.75)	2.65	1.73	
5d				
1st	3.61 ± 0.14 (3.17–4.03)	3.62	3.97	0.000
2nd	3.33 ± 0.05 (3.19–3.43)	3.34	1.42	
Ld m-d				
1d				
1st	0.79 ± 0.06 (0.65–0.92)	0.8	7.58	0.000
2nd	0.55 ± 0.05 (0.42–0.65)	0.55	8.98	
2d				
1st	1.46 ± 0.06 (1.32–1.6)	1.47	4.26	0.000
2nd	1.22 ± 0.05 (1.09–1.32)	1.22	4.02	
3d				
1st	2.14 ± 0.06 (2.0–2.29)	2.15	2.99	0.000
2nd	1.89 ± 0.05 (1.75–1.99)	1.89	2.63	
4d				
1st	2.82 ± 0.07 (2.68–2.98)	2.89	2.33	0.000
2nd	2.56 ± 0.05 (2.42–2.66)	2.56	1.96	

Table 2 (*continued*)

Parameters	Mean ± SD, mm (minimum-maximum)	Median, mm	Cofficient of variation (%)	*p*
5d				
1st	3.49 ± 0.07 (3.33–3.67)	3.50	1.99	0.000
2nd	3.24 ± 0.05 (3.1–3.34)	3.24	1.59	
Maxillary molars (1st and 2nd)				
mb-db				
1d				
1st	2.09±0.08 (1.84–2.23)	2.09	3.59	2.256
2nd	2.09±0.07 (1.84–2.23)	2.08	3.19	
2d				
1st	1.65 ± 0.07 (1.49–1.78)	1.65	4.34	0.000
2nd	1.51 ± 0.06 (1.4–1.64)	1.51	3.83	
3d				
1st	2.15 ± 0.08 (1.99–2.3)	2.15	3.64	2.445
2nd	2.05 ± 0.06 (1.94–2.18)	2.05	3.02	
4d				
1st	2.69 ± 0.09 (2.5–2.89)	2.69	3.40	
2nd	2.60 ± 0.06 (2.48–2.73)	2.60	2.37	2.336
5d				
1st	2.72 ± 0.09 (2.54–2.93)	2.72	3.40	1.747
2nd	2.64 ± 0.06 (2.53–2.78)	2.65	2.35	
db-pal				
1d				
1st	1.66 ± 0.04 (1.56–1.76)		2.61	0.000
2nd	1.42 ± 0.07 (1.27–1.55)		4.66	
2d				
1st	2.70 ± 0.05 (2.57–2.8)		1.73	0.000
2nd	2.45 ± 0.06 (2.31–2.59)		2.58	
3d				
1st	1.65 ± 0.07 (1.49–1.78)		4.34	0.000
2nd	1.42 ± 0.07 (1.27–1.55)		4.66	
4d				
1st	4.02 ± 0.10 (3.83–4.21)		2.54	0.000
2nd	2.45 ± 0.06 (2.31–2.59)		2.58	
5d				
1st	5.31 ± 0.10 (5.14–5.51)		1.94	0.000
2nd	1.42 ± 0.07 (1.27–1.55)		4.66	
mb-pal				
1d				
1st	2.09 ± 0.08 (1.84–2.23)		3.59	0.806
2nd	2.09 ± 0.07 (1.95–2.25)		3.19	

Table 2 (*continued*)

Parameters	Mean ± SD, mm (minimum-maximum)	Median, mm	Cofficient of variation (%)	*p*
2d				
1st	2.74 ± 0.06 (2.49–2.89)		2.26	0.464
2nd	2.75 ± 0.07 (2.64–2.9)		2.42	
3d				
1st	4.09 ± 0.07 (3.84–4.23)		1.67	0.597
2nd	4.09 ± 0.07 (3.96–4.25)		1.71	
4d				
1st	5.16 ± 0.12 (4.12–5.33)		2.29	0.219
2nd	5.14 ± 0.16 (4.38–5.33)		3.05	
5d				
1st	6.51 ± 0.13 (5.47–6.69)		1.93	0.689
2nd	6.50 ± 0.16 (5.73–6.7)		2.43	

Bd m-d – distance between mesial and distal root from buccal side; Ld m-d – distance between mesial and distal root from lingual side; mb-db – distance between mesiobuccal and distobuccal root; db-pal – distance between distobuccal and palatal root; mb-pal – distance between mesiobuccal and palatal root; 1d – first level of measurement; 2d – first level of measurement; 3d – third first level of measurement; 4d – fourth first level of measurement; 5d – fifth first level of measurement.

There was a statistically significant difference between the RC depth of the first and second molars, both at the buccal and lingual side. However, there was no proper distribution at each level in spite of the fact whether the RC depth was higher at the buccal or lingual side. The RC depth of distal roots, apically from the furcation, was the smallest one; the highest value of the RC depth was obtained at the buccal side of the mandibular second molar (Table 3). The mean values of the RC depth of maxillary molars were considerably lower than concavity of mandibular molars and did not exceed 2.74 ± 0.36 mm. RC was not found at the palatal root of maxillary molars, i.e., apically from the furcation (Table 3).

Table 3

The depths of root concavity of mandibular and maxillary molars

Parameters	Mean ± SD, mm (minimum-maximum)	Median, mm	Cofficient of variation (%)	*p*
Mandibular molars (1st and 2nd)				
BKF				
1st	1.02 ± 0.07 (0.85–1.34)	1.01	1.76	0.000
2nd	0.93 ± 0.19 (0.62–1.56)	0.89	1.84	3.6
LKF				
1st	0.88 ± 0.08 (0.74–1.28)	0.88	2.41	0.000
2nd	1.19 ± 0.21 (0.78–1.72)	1.19	7.06	0.000
BNF				
1st	3.28 ± 0.02 (3.22–3.38)	3.28	3.35	0.000
2nd	3.82 ± 0.33 (3.1–4.61)	3.79	5.62	0.000
LNF				
1st	3.71 ± 0.02 (3.65–3.74)	3.71	15.48	0.000
2nd	3.25 ± 0.44 (2.48–4.23)	3.12	17.39	3.02

Table 3 (*continued*)

Parameters	Mean ± SD, mm (minimum-maximum)	Median, mm	Cofficient of variation (%)	*p*
m root				
1st	0.49 ± 0.02 (0.46–0.54)	0.49	13.87	0.000
2nd	0.55 ± 0.06 (0.43–0.68)	0.54	15.01	0.49
d root				
1st	0.71 ± 3.37 (0.26–2.8)	0.29	27.42	0.000
2nd	0.25 ± 0.04 (0.18–0.33)	0.26	21.65	0.18
Maxillary molars (1st and 2nd)				
KF				
1st				
Mc	0.62 ± 0.10 (0.35–0.92)	0.63	15.48	2.33
Dc	0.53 ± 0.09 (0.25–0.78)	0.53	17.39	0.000
Bc	0.69 ± 0.10 (0.41–0.99)	0.7	13.87	0.000
2nd				
Mc	0.55 ± 0.08 (0.4–0.71)	0.53	15.01	0.000
Dc	0.34 ± 0.09 (0.17–0.52)	0.36	27.42	0.000
Bc	0.44 ± 0.09 (0.21–0.61)	0.44	21.65	0.000
NF				
1st				
Mc	2.74 ± 3.60 (2.36–42.0)	2.42	13.21	0.85
Dc	1.18 ± 0.02 (1.13–1.22)	1.17	1.76	0.000
Bc	2.71 ± 0.05 (2.21–2.76)	2.71	1.84	0.000
2nd				
Mc	2.68 ± 0.06 (2.48–2.78)	2.69	2.41	0.75
Dc	0.87 ± 0.06 (0.74–0.98)	0.87	7.06	0.000
Bc	1.83 ± 0.02 (1.7–1.96)	1.82	3.35	0.000
AF				
1st				
MBc	0.35 ± 0.02 (0.29–0.4)	0.35	5.62	0.000
DBc	0.03 (0–0.06)	0.03	60.45	0.000
PALc	0	0	0	0.000
2nd				
MBc	0.28 ± 0.02 (0.14–0.4)	0.29	20.15	0.000
DBc	0.04 (0–0.1)	0.03	55.10	0.000
PALc	0	0	0	0.000

BKF – depth of buccal root concavity coronally from the furcation; LKF – depth of lingual root concavity coronally from the furcation; B NF – depth of buccal root concavity on the roof of the furcation; LNF – depth of buccal root concavity on the roof the furcation; m root – depth of mesal root concavity (apically from the furcation); d root – depth of distal root concavity (apically from the furcation); KF – depth of root concavity coronally from the furcation from the Mc - mesial, Dc - distal and Bc - buccal side; NF – depth of root concavity on the roof of the furcation; AF-NF – depth of root concavity apically of the furcation; MBc – depth of mesiobuccal root concavity (apically from the furcation); DBc – depth of distobuccal root concavity (apically from the furcation); PALc – depth of palatal root concavity (apically from the furcation).

Discussion

Anatomical features of furcation area may cause initiation and persistence of periodontal disease. The FI tooth leads to more difficult diagnosis and makes the treatment outcome less predictable. Johansson et al. [26] reported that the molars with FI were more frequently lost after 13–16 years of periodontal therapy compared to the molars without FI. It has been indicated that the teeth with FI respond less favorably to the conventional periodontal therapy compared to the noninvolved molars, or the one-rooted teeth [8]. The researches on the incidence of exacerbation over a two-year period following the nonsurgical periodontal therapy pointed that the probing attachment loss was two to three times more frequent in furcation defects compared to nonfurcation areas [27]. As for the individuals aged 40 and more years, every second molar was affected by the advanced periodontal destruction (Class II-III) in at least one furcation site [28]. Furthermore, the prevalence of molars with FI was found to be higher in the maxilla than in the mandible. The most commonly affected tooth site was the distal aspect of the first maxillary molars [29].

RTL has important impact on the pathogenesis of periodontal disease. This is one of the key anatomical factors that make molars particularly susceptible to periodontal disease [30]. If a root trunk is shorter, it will lead to the earlier occurrence and development of diseases; however, it will be easier to instrument a furcation lesion [31–34]. On the other hand, a long root trunk protects furcation from periodontal disease involvement in the initial stage of periodontitis [24]. If a furcation is affected, the prognosis is poorer for higher RTL, because the access for instrumentation is hampered [25]. Additionally, the FI molar with the short roots indicates the reduced chance of repair after the periodontal therapy and it could not be a candidate for the root apicectomy because the periodontal support of these teeth is lost in proportion with the furcation invasion [18, 24, 35]. Horwitz et al. [33] concluded that a long root trunk and wide FE decreased the chance of successful periodontal treatment.

According to our measurement, the highest RTL value of 7.57 ± 0.86 mm was found at the distal side of maxillary first molars and 6.94 ± 0.85 mm at the lingual side of mandibular first molars. On the other hand, the minimum value of the buccal side of mandibular first molar was 2.26 ± 0.71 mm and maxillary first molar 2.45 ± 0.70 mm at the distal side. This means that at the beginning of periodontitis, the consequences of furcation involvement may occur at the probing depth of 3–4 mm [36]. Furthermore, we found higher root trunks of the first maxillary molars compared to the second ones. It was opposite with the mandibular molars on the buccal side, where the second molars were of a higher RTL value than the first ones, which corresponded to the results of the study of Sanz et al. [37]. The results of our study regarding RTL also corresponded to the findings of Hou and Tsai [19] and Plagmann et al. [36]. They showed significantly higher RTL at the oral sides than at the buccal sides of mandibular molars as well as at the approximal sides than at the buccal sides of maxillary molars. The mandibular molars generally have shorter root trunks than the maxillary molars [38].

The prognosis for molars with short root trunks and more divergent roots is better when root resection is applied [39]. A short root trunk and a wide diameter of the furcation entrance are criteria for a tunnel preparation. Such a procedure is a part of resective furcation therapy used to enable a patient to manage postoperative plaque properly [18].

RL is directly related to the amount of a tooth attachment support [1]. In the present study, the mean RL of mandibular first molars was significantly smaller than that of the second ones, which matched the results obtained by Roussa [25] and Bower [40], while the RL of the maxillary molars showed different results. The highest mean value was measured for the palatal root, unlike the study of Roussa [25] which showed the highest RL of distobuccal root [40]. The distobuccal root of maxillary first molars and the distal root of the mandibular first molars had the smallest RL [40]. Therefore, when all other factor are identical, these roots are the first to be removed when root resection procedures are considered.

The diameter of the furcation entrance is another important factor. Svärdström and Wennström [28] found the highest frequency of FI at the distal side of maxillary first molars (53%), while the lowest frequency was with the mesial aspects of maxillary second molars (20%). The complexity of the area morphology after the attachment loss creates a favorable environment for bacteria plaque retention and contributes to the pathogenesis of the periodontal destruction [24, 28].

Proper instrumentation of furcation defects has always been a challenge for dentists due to the limited accessibility through furcation entrances. The blades of periodontal manual instruments, curettes have to be of a width that would produce a smooth and biologically acceptable surface, which would allow satisfactory healing [41, 42]. Various studies regarding the relationship of FE and blade widths confirmed such difficulty in the periodontal therapy of molar furcations [23, 40]. The diameter of FE was < 0.75 mm in about one half of the measured teeth; however, in more than 80% of the teeth such entrance diameters were < 1 mm and the active tip of an instrument (e.g.: Gracey curette), being 0.95–1.2 mm wide, does not fit to the furcation area [23, 40, 43, 44].

The results of our study showed that the mean values of FE for the mandibular molars, except buccal FE of the first ones, were lower than 0,75 mm as well as the buccal FE of the maxillary molars. Interestingly, the buccal FE of maxillary tooth was the narrowest. The mesial and distal FE of maxillary molars were higher than 1mm, except distal FE of the second molar.

A recent study of the radiographic characteristics of FI showed that narrow FE can have better outcome after the nonsurgical periodontal therapy. It probably resulted from the lower exposure to contaminants and less root irregularities [45]. With reduced root separation, the use of hand instruments cannot ensure effective root surface instrumentation in the furcation as a basis for successful healing. An ultrasonic scaler is smaller than curette tips and it is recommended for the periodontal treatment of furcation involvement [46, 47]. In such cases, the use of special instruments, e.g., diamond-coated air scaler tips for the odontoplastic method are recommended [48].

Regarding the regenerative therapy, Pepelassi et al.[49] showed that the distance between the roots of 2 mm, or greater ensures more favorable regenerative healing. The results from our study showed that DBR greater than 2 mm was at the third level of measurement, i.e., 3 mm from the FE, except for the first maxillary molar. It had such a distance even at the first level (between the mesiobuccal and palatal root). However, it was concluded that higher root divergence was associated with a larger furcation defect, which may be accompanied with the reduced horizontal bone gain, furcation closure and favorable regenerative outcome [14]. Moreover, Pontoriero, et al. [51] stated that the furcation width at radicular separation area greater than 4 mm² and the FE height of 3 mm, or greater failed to heal the complete defect closure. This means that it should be added to the list of making treatment decision whether the regenerative therapy is indicated or not in a specific region of FI teeth.

In case of a short root trunk, the occurrence of developmental grooves and trunk surface concavities are other factors to be considered as the contributors to the outcome of nonsurgical or regenerative periodontal therapy [52].

Interestingly, the RC of maxillary molars had the significantly higher measured values of mandibular molars. The palatal root demonstrated complete absence of the concavity. Lu [53] reported that the depth of root trunk developmental concavities was variable in 94% furcations. Our results do not correspond to those of Roussa [25] and Dunlap and Gher [20] study, who showed larger concavities at the buccal aspects both for the first and the second mandibular molar. The mean value of the RC of second mandibular molars was higher at the lingual aspects.

The RC increases the attachment area of the tooth, thus making it resistant to the torque forces. On the other hand, curettes alone would most probably fail to achieve adequate preparation of deep concavity of furcation. Additionally, the concavities may hamper complete coverage of root surfaces by membrane. Lu [53] measured the concavities at the level of 1–2 mm below the cement-enamel junction and found concavities ranging from 0.00 to 2.25 mm. Based on these observations, the author concluded that in the majority of molars, the subgingival application of a guided tissue membrane being 1–2 mm below the cement-enamel junction could not ensure complete adaptation of furcation defects.

According to the study of Schwendicke et al. [54] "the periodontal treatments aimed at tooth retention were found to be more effective and less costly than tooth replacement with implant supported crowns (ISCs) in the treatment of furcation class II/III. Despite long-term retention of FI molars, different intervals of supportive periodontal treatment and even surgical procedures, the costs were still less than implant supported crowns with the exception of root resection".

Conclusion

The value for the buccal RTL of mandibular first molars was the lowest, which could lead to an early appearance of FI. The buccal FE was the narrowest in the maxillary molars, and the distal FE was the most apically positioned, which could be rather challenging to be diagnosed. The mean value of RC of the mandibular second molar was the highest one. The palatal roots of the maxillary first and second molars were without concavities. The total of 468 teeth indicate the variability of furcation morphology, having thus considerable influence on the etiology and severity of periodontitis as well as on the therapeutic success and possible recurrence of the disease or disease progression.

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Psychological characteristics in patients with non-cardiac chest pain

Psihološke karakteristike bolesnika sa bolom u grudima bez srčanog uzroka

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Abstract

Background/Aim. Chest pain of no heart origin resembles angina and when none medical reason is found, the patients are referred to psychiatrist for further assessment. The aim of this reserch was to determine psychological characteristics of the patients with non-coronary chest pain (NCCP), difference compared to the coronary patients and the predictive value of those parameters for NCCP. **Methods.** Fourty consecutively recruited patients without a diagnose of heart disease (NCCP group) were examined and compared to 45 coronary patients (C group). For psychiatric diagnose, the Mini-International Neuropsychiatric Interview (MINI) was used. Psychological symptoms were assessed by the Symptom Checklist-90-Revised (SCL-90R), exposure to life events was scored by the Holms &Rahe Scale and levels of anxiety and depressiveness by the Back Anxiety Inventory and Back Depression Inventory. The statistical analysis was done by using the software package SPPS17. The Student's-*t* test and χ^2-test were used for estimating more difference between groups while ANOVA determined parameters associated with NCCP. **Results.** The NCCP patients were younger (33.40 ± 5.43 vs. 48.37 ± 6.43, $p < 0.001$), more anxious (20.47 ± 11.93 vs. 9.63 ± 3.86, $p < 0.001$), had more exposure to life events (102.03 ± 52.22 vs. 46.5 ± 55.08, $p < 0.001$) and were more distressed (41.37 ± 7.70 vs. 29.37 ± 5.67, $p < 0.001$), while coronary patients were more depressed and hostile. The regression analysis indicated that elevation in anxiety score for 1 point, means 25% of a higher chance [odds ratio (OR) = 1.25; 95% confidence interval (CI): 1.10–1.41] and elevation in the Life events score, means 2% of a higher chance that subject belonged to the NCCP group (OR = 1.02; 95% CI: 1.01–1.03). The younger subjects were more likely to have non-cardiac chest pain (OR = 0.58, 95% CI: 0.42–0.80). **Conclusion.** The results suggested that the patients with NCCP had none associated psychiatric disorder, but showed higher distress level, more exposure to negative life events and moderate anxiety level. Psychological help could be of a benefit to prevent possible psychiatric issues in young people with non-cardiac chest pain.

Key words:
coronary disease; chest pain; diagnosis, differential; risk factors; psychological tests; stress, psychological; anxiety; depression.

Apstrakt

Uvod/Cilj. Bol u grudima koji nije srčanog porekla često liči na anginozni i kada se ne pronađu medicinski uzroci, bolesnici se upućuju psihijatru radi dalje procene. Cilj istraživanja bilo je utvrđivanje psiholoških karakteristike bolesnika sa bolom u grudima bez koronarnog uzroka, razlika u poređenju sa koronarnim bolesnicima i prediktivnih vrednosti parametara za bol bez koronarnog uzroka. **Metode.** Konsekutivno je bilo regrutovano 40 bolesnika bez dijagnoze srčane bolesti (BDSB grupa) sa simptomima bola u grudima, koji su upoređeni sa 45 koronarnih bolesnika (K grupa). Za postavljanje dijagnoze psihijatrijske bolesti korišćen je Mini-internacionalni neuropsihijatrijski intervju (MINI), za procenu psiholoških simptoma Upitnik liste simptoma-90-revidirani (SCL-90R upitnik), za procenu izloženost životnim događajima Holmas Rahe skala, a za procenu nivoa anksioznosti i depresivnosti Bek–ov upitnika za anksioznost i Bek-ov upitnik za depresivnost . Statistička analiza rađena je pomoću SPPS 17, a korišćeni su Student-ov *t*-test i χ^2 test za utvrđivanje razlike između parametara u grupama. ANOVA je upotrebljena radi određivanja parametara koji su povezani sa bolom u grudima bez koronarnog uzroka. **Rezultati.** Bolesnici u BDSB grupi bili su mlađi (33,40 ± 5,43

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vs 48,37 ± 6,43, $p < 0,001$), anksiozniji (20,47 ± 11,93 *vs* 9,63 ± 3,86, $p < 0,001$), više izloženi životnim događajima (102,03 ± 52,22 *vs* 46,5 ± 55,08, $p < 0,001$) i imali su viši nivo distresa (41,37 ± 7,70 *vs* 29,37 ± 5,67, $p < 0,001$), dok su koronarni bolesnici bili više depresivni i hostilni. Regresiona analiza je pokazala da porast skora anksioznosti za 1 poen, znači 25% veću šansu da subjekt pripada BDSB grupi [*odds ratio* (OR) = 1,25; 95% interval poverenja (IP): 1,10–1,41] i porast skora životnih događaja znači 2% veću šansu da bolesnik pripada BDSB grupi (OR = 1,02; 95% IP: 1,01–1,03). Mlađi ispitanici imali su veću šansu da pripadaju BDSB grupi (OR = 0,58, 95% CI: 0,42–0,80). **Zaključak.** Bolesnici BDSB grupe nisu imali udruženi psihijatrijski poremećaj, ali su imali viši nivo distresa, izloženost životnim događajima i umereni nivo anksioznosti. Psihološka pomoć mladim ljudima sa bolom u grudima bez srčanog uzroka, mogla bi biti korisna u cilju prevencije mogućih psihijatrijskih poremećaja.

Ključne reči:
koronarna bolest; grudi, bol; dijagnoza, diferencijalna; faktori rizika; psihološki testovi; stres, psihički; anksioznost; depresija.

Introduction

Pain is a main medical symptom motivating people to have a medical examination. If uncomfortable sensations are in the chest, a cardiologist is always involved. Physical, biochemical, para-clinical and invasive diagnostic procedures are used to diagnose cardiovascular disease (CVD), or other medical cause for chest pain [1]. If the results are negative and sensations are not of somatic origin, it is possible that psychological factors play a role in such clinical manifestation [2]. Carefully taken medical history can sometimes discover emotional distress, precipitating oppressive squeezing or pressure [3]. In these cases, the patients are referred to a psychiatrist, for further assessment and treatment. Some researches in this area consider every chest pain a sign of subclinical atherosclerosis (syndrome X), even when the invasive techniques (percutaneuos angiography) do not confirm atherosclerosis of heart arteries [4, 5]. However, the patients prone to anxiety reactions to stressful situations, have somatic sensations due to surges in catecholamines. Located in the chest, the sensations reinforce anxiety and fear of death, leading to numerous claims for cardiologic checks, despite negative results for CVD. On the other hand, it is well-known, that general anxiety disorder is a risk factor for experiencing acute cardiac events in the CVD patients [6].

In consultative psychiatric practice, we have examined the patients without a CVD diagnose , but with complains of atypical, pain-like sensation in their chest. In many cases, we could not set psychiatric diagnose since their symptoms did not match any disorder according to the International Classification of Diseases-10 (ICD-10) [7].

The aim of this research was to determine the psychological characteristics of the patients with non-coronary chest pain (NCCP), the differences comparing the coronary patients and the predictive value of NCCP parameters.

Methods

Subjects

The cross-section study was performed in the Clinic for Mental Health Protection in Niš with 40 consecutively recruited patients with NCCP. Inclusive criteria were absence of diagnosed organic cause of chest pain and psychotic disorder. The subjects were referred to a psychiatrist for the further examination, after heart disease and other medical reasons for chest pain were ruled out by the complete physical and at least twice repeated the cardiologic paraclinical examination: treadmill test, echocardiography, electrocardiography, biochemical analyses and markers for myocardial ischemia in the Clinic of Cardiology, Clinical Centre in Niš. Coronary angiography was not performed due to the previous negative results, but neurological, gastroenterological and surgical check-ups were also done prior to the psychiatric evaluation. The pain characteristics were obtained from the medical data. The diagnostic algorithm included the description of pain, intensity (scale from 1–10), location, duration of sensation, circumstances of pain occurrences and what stops the pain. All subjects diagnosed with organic pain (esophageal reflux, ulcers, diaphragm hernia, degenerative process in vertebrae and costal-vertebral joints, rheumatism, pulmological and pleural conditions) were excluded from further psychiatric evaluation. The control group (C group) consisted of 45 patients with coronary disease confirmed by suffered myocardial infarction and coronary angiography in the previous three years. The exclusion criteria were presence of other medical condition associated with chest pain. From the pool of 105 previously recruited subjects, only 40 subjects from the NCCP and 45 subjects from the C group fulfilled inclusive criteria and completed the questionnaires.

The psychiatric evaluation was conducted in the outpatient setting during one-year period.

All patients gave their written consent to participate after receiving information about the study. The local Ethics Committee Permission and institutional approval were obtained.

Instruments

The demographic and data of risk health behavior were collected from the medical records. Risky alcohol consumption was determined as more than 7 standard drinks per week (14 grams of ethyl alcohol). The smoker status meant smoking cigarettes now or ever. The presence of physical activity meant a physical effort at least three times a week, lasting for 1 hour and the absence means sedentary lifestyle [8].

For the psychiatric diagnose, we used the Mini International Neuropsychiatric Interview [9], (MINI). Two psychiatrists performed evaluation for each patient and compared results with criteria at ICD-10 manual.

The presence of psychological symptoms in the previous week as well as the level of actual distress were assessed by the Symptom Checklist-90-Revised (SCL-90R), a questionnaire containing 90 items, grouped in 9 subscales describing 9 dimensions of psychopathology. Higher level of each, indicated more prominent characteristics [10, 11]. The indicators of actual disstress level were calculated from the scales : Global Severity Index (GSI), Positive Symptom Total (PST) and Positive Symptom Distress Index (PSDI). Every dimension score > 63, meant that it was of a clinical significance.

The Back Anxiety Inventory (BAI) was used to estimate intensity of anxiety [12]. It was a multiple-choice, self-reported questionnaire of 21 items, scored on Likert scale from 0 (not at all) to 3 (severely expressed). Cut-off score for clinically expressed anxiety was 8 points. The instrument was very reliable, Cronbash's $\alpha = 0.92$, [95% confidence interval (CI): 0.89–0.95].

The Back Depression Inventory (BDI) measured depressiveness. It consisted of 21 items, each scored on the four-point Likert scale from 0–3 with the total score ranging 0–63. Cut-off score for clinical significant depression was 10. The higher score indicated more severe depressiveness [13]. The Cronbach`s alfa coefficient for the sample was 0.68 (95% CI: 0.53–0.80), meaning a good reliability of the instrument for the sample.

The Holms-Rahe (H-R) scale was self – rating inventory measuring experience of life events in the previous year. The score ≥ 100 shows the predisposition for an anxious reaction; more than 150 points mean 30% of chance to somatic breakdown due to distress [14].

Statistical analysis

The data collected through a direct contact with the patients, or from a patient's history (total n = 85) were presented as the frequency distribution tables expressed as percentages and analyzed using the SPSS version 17.0 (IBM Corp, 2007). The existence of any statistical difference between the groups was defined using the Student's *t*-test and χ^2-test for parametric and non-parametric parameters, respectively. The ANOVA regression analysis was performed to determine the parameters associated with non-cardiac chest pain, all the statistically significant parameters found by the univariate analysis were introduced into the multivariate regression analysis in order to identify the independent factors associated with non-cardiac chest pain. The probability values (*p*) that were less or equal to 0.05 were considered statistically significant. In order to estimate the reliability of the psychometric tests (BAI and BDI), the Cronbach's alpha was calculated.

Results

Fourty NCCP patients were compared to 45 coronary patients (C group) for the demographic, biological and risk health behavior characteristics.

The groups were similar by all demographic parameters except the age, frequency of hypercholesterolemia, diabetes mellitus and hypertension. The subjects with NCCP were significantly younger, had less biological risk factors compared to the coronary patients (Table 1). There were no differences in the lifestyle characteristics, between then groups.

Psychological characteristics and distress levels

The psychological symptom pattern assessed by the SCL-90R indicated depression and hostility among the coronary patients as significantly higher. The distress measures: GSI and PSDI were significantly higher in the NCCP group, indicating emotional disturbances experienced by a patient with NCCP (Table 2).

Table 1
Difference in the demographic characteristics, health risk behavior and biological factors

Variables	Groups		*p*
	NCCP (n = 40) n (%)	C (n = 45) n (%)	
Females	24 (60.00)	25 (55.55)	ns
Married	36 (90.00)	33(73.33)	ns
Education 8 years	6 (15.00)	9 (20.00)	ns
Education 12 years	30 (75.00)	25 (55.55)	ns
Education > 12 years	3 (7.50)	8 (17.77)	ns
Employment	21 (52.50)	29 (64.44)	ns
Age (years), mean ± SD	33.40 ± 5.43	48.37 ± 6.43	< 0.001
Risky alcohol consumption	3 (7.50)	8 (17.77)	ns
Smoking	13 (32.50)	18 (40.00)	ns
Sedentary lifestyle	21 (52.50)	18 (40.00)	ns
Hypercholesterolemia	8 (20.0)	34 (75.55)	< 0.001
Diabetes mellitus	2 (5.00)	12 (26.66)	< 0.05
Hypertension	13 (32.50)	28 (62.22)	< 0.05

NCCP – non-coronary chest pain patients; C – coronary patients; SD – standard deviation; ns – non significant.

Table 2

Difference in psychological dimensions between the groups

SCL-90R dimensions	Groups		*p*
	NCCP (n = 40) mean ± SD	C (n = 45) mean ± SD	
Somatization	60.63 ± 9.33	59.93 ± 4.58	ns
Obsessive-Compulsive	49.03 ± 11.95	50.27 ± 6.46	ns
Interpersonal Sensitivity	46.27 ± 10.77	49.63 ± 6.33	ns
Depression	48.33 ± 9.85	58.60 ± 4.70	< 0.001
Anxiety	58.07 ± 11.69	61.77 ± 5.60	ns
Hostility	50.63 ± 9.47	57.40 ± 5.03	< 0.01
Phobic anxiety	54.37 ± 9.15	54.43 ± 7.49	ns
Paranoid ideation	46.77 ± 9.77	46.23 ± 6.48	ns
Psychoticism	44.63 ± 11.04	41.27 ± 6.19	ns
Global Severity Index	41.37 ± 7.70	29.37 ± 5.67	< 0.001
Positive Symptom Distress Index	21.03 ± 20.92	0.20 ± 0.61	< 0.001
Positive Symptom Total	43.47 ± 25.51	43.97 ± 13.34	ns

SCL-90R – the Symptom Checklist-90-Revised; NCCP – non-coronary chest pain patients; C – coronary patients; SD – standard deviation; ns – non-significant.

Levels of anxiety, depressiveness and life events exposure

The anxiety level was moderate in the NCCP group and significantly different from the anxiety in coronary patients, which was in the normal range. The average score of life events was also significantly higher in the patients with NCCP, while the level of depressiveness was without a significant difference and below the cut-off limits in both groups (Table 3).

Predictive values of psychological parameters for cardiac chest pain

The univariate logistic regression showed that a patient with the increased levels of depression, hostility and distress had significantly higher chance to have coronary chest pain (Table 4).

Table 3

Difference in anxiety, depressiveness and exposure to life events between the groups

Psychological variables	Groups		*p*
	NCCP (n = 40) mean ± SD	C (n = 45) mean ± SD	
Anxiety (BAI)	20.47 ± 11.93	9.63 ± 3.86	< 0.001
Depressiveness (BDI)	9.60 ± 5.01	7.73 ± 1.91	ns
Holms-Rahe scale	102.03 ± 52.22	46.5 ± 55.08	< 0.001

NCCP – non-coronary chest pain patients; C – coronary patients; BAI – Back Anxiety Inventory; BDI – Back Depression Inventory; SD – standard deviation; ns – non-significant.

Table 4

Association of psychological dimensions with cardiac chest pain

SCL-90R	OR	95% CI for OR		*p*
		lower	upper	
Somatization	1.01	0.94	1.08	ns
Obsessive-Compulsive	0.97	0.93	1.04	ns
Interpersonal Sensitivity	0.96	0.90	1.02	ns
Depression	0.82	0.73	0.91	< 0.01
Anxiety	0.95	0.90	1.01	ns
Hostility	0.88	0.80	0.95	< 0.01
Phobic anxiety	0.99	0.93	1.06	ns
Paranoid ideation	1.01	0.94	1.07	ns
Psychoticism	1.04	0.98	1.11	ns
Global Severity Index	0.76	0.66	0.87	< 0.001
Positive Symptom Distress Index	0.83	0.64	1.06	ns
Positive Symptom Total	0.99	0.97	1.02	ns

SCL-90R –Symptom Checklist-90-Revised; ns – non-significant; OR – odds ratio; CI – confidence interval.

Table 5
Association of age, emotional reactions, biological risk factors and life events score with non-cardiac chest pain

Psychological and biological variables	OR	95% CI for OR		*p*
		lower	upper	
BAI Anxiety	1.25	1.10	1.41	< 0.01
BDI Depressiveness	1.17	0.97	1.39	ns
Holms-Rahe Scale	1.02	1.01	1.03	< 0.01
Age	0.58	0.42	080	< 0.01
Hypercholesterolemia	0.08	0.02	0.30	< 0.01
Diabetes mellitus	0.09	0.01	0.82	< 0.05
Hypertension	0.29	0.09	0.95	< 0.05

OR – odds ratio; CI – confidence interval; BAI – Back Anxiety Inventory; BDI – Back Depression Inventory; ns – non-significant.

The prediction factors of non-coronary chest pain

The regression analysis indicated that an elevation in the anxiety score for 1 point, meant 25% higher chance that the subject belonged to the NCCP group [odds ratio (OR) = 1.25; 95% CI: 1.10–1.41]. An elevation in the life events score meant that there were 2% higher chance that the chest pain was not the coronary one (OR = 1.02; 95% IP: 1.01–1.03) (Table 5).

The results showed that the younger subjects were more likely to have NCCP (OR = 0.58, 95% CI: 0.42–0.80).

All factors that were statistically significant according to the univariate analysis were introduced into the multivariate analysis, but no parameter was found to be significant (data not presented). However, the patients' age and anxiety level were near the statistically significant value [$p = 0.0517$ (OR = 0.892) and for anxiety, it was found that $p = 0.0581$ (OR = 1.025), respectively]. A limited number of patients in the study were probably a reason for the absence of statistical significance, but these parameters could possibly be associated with NCCP.

Discussion

This study focused on the forty patients with angina-like sensations, free of CVD. Their uncomfortable sensations were varying in their location in the chest, duration, description and intensity. They were not associated with physical strain, food intake or change in body position. It occurred more than twice a week, sometimes every day and lasted for hours. The subjects described their pain like squeezing, burning, pressure, chest barrier, sharp needle sticks. The psychiatric anamnesis indicated that the negative life events and emotional distress preceded the sensations, but it also appeared during the night and at rest and everyday situations. The pain like a sensation usually diminished spontaneously, or with using anxiolitics. In relevant literature, it was reported that about 20% of those seeking help from cardiologists had normal coronary arteries, confirmed by coronary angiography [15]. They underwent numerous examinations due to their fear of undiscovered heart disease. When there was no evidence of CVD, or other organic cause (esophageal reflux, esophageal motor dysfunction, musculoskeletal issues) a psychiatrist was involved in further diagnose and treatment. In our NCCP patients, we noticed a constant worry that a heart attack could occur, and they continued to take antianginal medications despite a lack of diagnose. The psychiatric examination included the unstructured clinical interview, SCL-90R questionnaire and heteroanamnesis' data from family members. Although their symptoms resembles somatoform and anxiety disorders, all criteria for psychiatric diagnose were not present. The most frequent symptoms were emotional and muscular tension, difficulties falling asleep, emotional irritability, conflicts in relationship with important persons and hypochondriac concerns.

In order to explore main difference from the CVD patients considering the psychological factors, the NCCP group was compared to the patients with the established coronary artery disease (previous myocardial infarction or arteriography, with diagnose of angina). The NCCP group consisted of younger patients, as expected. The neurotic fear of illness and anxiety syndromes usually occur in young adults, while atherosclerosis and myocardial ischemia are more frequent in middle age and elderly persons, as confirmed in literature [16, 17]. Health-risk behaviors were equally present in both groups, although we expected to be more frequent in the C group. Nicotine has a toxic effect on endothelium, accelerating atherosclerosis. Smoking increases risk or future cardiac event and mortality rate in older patients as confirmed on the Korean sample in a study of Ahn et al. [16] from 2016. Also, a lack of physical activity and a risk of alcohol consumption, are recognized pathological factors for poor medical outcome and health-related quality of life among the individuals without a diagnose of cardiovascular disease. These self-harm behaviors are some psychological mechanisms for overcoming negative emotions and are associated with the symptoms of anxiety [18–20], that was also present in our sample of subjects with chest discomfort. The educational preventive measures might reduce the chance of future CVD, as shown in prospective 20 years long study of youth, where change in lifestyle behavior was linked to diminishing chance for heart disease in adulthood [18].

In both groups, no psychiatric diagnose was found according to the MINI and ICD-10 criteria. Other researches considered NCCP as somatic expression of anxiety, or panic attack [19, 20], or a symptom of other somatic illness [21]. The subjects in the NCCP group had somatic complains, but within the psychological dimensions of depression and hos-

tility, measured by the SCL-90R, questionnaire, they were more prominent in the K group (CVD) patients. This was in line with some other findings [21, 22]. In these researches, depressiveness and hostility were spotted as toxic emotions, playing a role at a psycho-physiologic pathway for coronary artery disease and acute cardiac events.

Other eight psychological symptoms (SCL-90R) were equally represented, but none of them reached clinically significant level. This finding confirmed absence of psychiatric disorders, not only in the CVD patients, but also in the subjects with NCCP as well. This was not in line with the study of Campbell et al. [1], where a psychiatric diagnose of NCCP included: anxiety disorder (AD), panic disorder (PD), somatoform disorder (SD) and mayor depressive disorder (MDD). Clinically, inner tension dominated in our subjects. The most of them had the anxiety, or somatization symptoms, hypochondriac preoccupations and dysphoria, lasting from few weeks to few months, without fullfield criteria for psychiatric diagnose. In the NCCP group, the level of distress was significantly higher (GSI and PSDI), which indirectly confirmed psychological mechanism associated with somatic sensations. The similar findings explained physical symptoms as somatic expression of distress in the alexthymic persons [23, 24]. In the NCCP group, the level of anxiety was moderate and the score on the H-R scale of life events was > 100. This finding indicated coexistence of physical sensations, anxiety symptoms and exposure to stress life events being different from the coronary patients who had levels of anxiety, depression and life events score within a normal range. Such result differs from other researches in which almost 20% of patients after myocardial infarction suffered from some form of depressive disorder [25], which had a negative influence on prognosis [25, 26]. In the C group, none of the patients asked for a psychological help after their coronary event.

Some researchers and cardiologists consider atypical chest pain as subclinical manifestation of CVD when associated with positive stress test [27]. The patients from the NCCP group, took medications prescribed by a cardiologist (beta-blockers, acetylsalicylic acid), despite the fact that CVD was not confirmed. We assumed their chest discomfort was in connection with the psychological characteristics. The regression analysis showed that the persons with the depression, hostility and global distress had a greater chance for non-cardiac chest pain. Exposure to undesirable life events in the previous year, moderate anxiety level and younger age were associated with NCCP, but none of the parameters had a predictive value in the multivariate analysis. All together, those psychological features and anxiety, coexisting with NCCP, indicated the pathophysiological mechanism of somatization related to NCCP [28]. It could be assumed that the persons with the negative affectivity (hostility, depression, anxiety, distress), without a psychiatric diagnose, expressed their inner tension on the somatic level. They could be in the psychosomatic double pathway: to develop psychiatric disorder, or heart disease in the future. They need psychological help to deal with their emotions efficiently in order to decrease chance for mental or cardiovascular disorder [29, 30].

There are several limitations of the generalizability of the findings in this study. A larger sample would be better to determine psychological features associated with sensations in chest without CVD. No psychological characteristics specific only for NCCP were present in the patients with heart disease. There was no follow-up to check if some of the features changed over time and whether some other cause of chest pain appeared. A longitudinal study is necessary to confirm a possible development of psychiatric or CVD disorders in our patients with non-cardiac chest pain. The findings would be more valuable if we compare our NCCP group results not only with coronary patients but with the healthy controls as well.

Conclusion

The results suggested that the patients who complained about chest pain without diagnose of cardiovascular disease, or other somatic illness, had none associated psychiatric disorder, but express a higher distress level, exposure to the negative life events in the previous year and moderate anxiety level. The patients with cardiovascular disease felt hostility and elevated depression. The younger age, the moderate anxiety and exposure to life events were predictive for non-cardiac chest pain. Psychological help could be of a benefit to prevent possible psychiatric issues in young people with non-cardiac chest pain.

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Left ventricle ejection fraction and strain derived by three-dimensional echocardiography are associated with exercise capacity in the patients with heart failure

Ejekciona frakcija leve srčane komore i miokardna deformacija dobijene trodimenzionalnom ehokardiogrfijom povezane su sa funkcionalnim kapacitetom bolesnika sa srčanom insuficijencijom

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Abstract

Background/Aim. Echocardiography represents the most commonly performed noninvasive cardiac imaging tests for the patients with heart failure (HF). The aim of this study was to assess the relationship between the exercise capacity parameters [peak oxygen consumption (VO_2) and the minute ventilation-carbon dioxide production relationship (VE/VCO_2)] and the three-dimensional speckle-tracking echocardiography (3D-STE) imaging of left ventricular (LV) function in the HF patients with the reduced LV ejection fraction (LVEF). **Methods.** This cross-sectional study included 80 patients with diagnosed ischemic LV systolic dysfunction (LVEF < 45%) divided into subgroups based on the proposed values of analyzed cardiopulmonary exercise testing (CPET) variables: VO_2 peak ≤ 15 mL/kg/min, VO_2 peak > 15 mL/kg/min, VE/VCO_2 slope < 36 and VE/VCO_2 slope ≥ 36. All patients underwent a physical examination, laboratory testing, two-dimensional (2D) and 3DE, and CPET. **Results.** LVEF, global longitudinal, circumferential, radial and area strains were significantly lower in the subgroups of subjects with a peak VO_2 less, or equal to 15 mL O_2/kg per min and with a VE/VCO_2 slope greater, or equal to 36 compared to the subgroups of subjects with a peak VO_2 greater than 15 mL O_2/kg per min and with a VE/VCO_2 slope less than 36. There was a significantly positive correlation between the peak VO_2 values and parameters of 3DE, and a significantly negative correlation between the VE/VCO_2 slope values and parameters of 3DE. **Conclusion.** The results of this study provide further evidence that the LV function can be noninvasively and objectively measured by 3D-STE. A significant correlation between examined parameters suggests that LVEF and strain derived by 3DE are associated with exercise capacity in the patients with HF.

Key words:
heart failure; myocardial contraction; echocardiography; ventricular function, left; exercise test; oxygen consumption.

Apstrakt

Uvod/Cilj. Ehokardiografija predstavlja jedan od najčešće izvođenih neinvazivnih testova analize bolesnika sa srčanom insuficijencijom. Cilj ove studije bio je da se utvrdi odnos između parametara funkcionalnog kapaciteta [maksimalna potrošnja kiseonika (VO_2), ventilatorni ekvivalent za ugljen-dioksid (VE/VCO_2)] i trodimenzionalne ehokardiografije (3D-STE) leve komore kod bolesnika sa srčanom insuficijencijom i smanjenom ejekcionom frakcijom leve srčane komore (LVEF). **Metode.** Ova studija preseka obuhvatila je 80 bolesnika sa dijagnostikovanom sistolnom disfunkcijom leve srčane komore ishemijske etiologije (LVEF < 45%) podeljene u podgrupe na osnovu predloženih vrednosti analiziranih parametara funkcionalnog kapaciteta: VO_2 ≤ 15 mL/kg/min, VO_2 > 15 mL/kg/min, VE/VCO_2 < 36 i VE/VCO_2 nagib ≥ 36. Svi bolesnici podvrgnuti su fizičkom pregledu, laboratorijskom testiranju, 2DE i 3DE, kao i ispitivanju funkcionalnog kapaciteta. **Rezultati.** Vrednosti LVEF, longitudinalne, cirumferentne, radijalne i površinske miokardne deformacije bile su značajno niže u podgrupama bolesnika sa vrednostima VO_2 ≤ 15 mL O_2/kg po min i VE/VCO_2 ≥ 36 u odnosu na bolesnike podgrupa sa VO_2 > 15 mL O_2/kg po min i VE/VCO_2 < 36. Uočena je značajna pozitivna korelacija između izmerenih vrednosti VO_2 i parametara 3DE, te značajna negativna korelacija između izmerenih vrednosti VE/VCO_2 i parametara 3DE. **Zaključak.** Dobijeni rezultati potvrđuju da se funkcija leve

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komore može neinvazivno i objektivno proceniti primenom 3DE. Uočena značajna korelacija između između ispitivanih parametara ukazuje na to da su LVEF i miokardna deformacija dobijeni 3DE povezani sa funkcionalnim kapacitetom bolesnika sa srčanom insuficijencijom.

Ključne reči:
srce, insuficijencija; miokard, kontrakcija; ehokardiografija; srce, funkcija leve komore; vežbanje, testovi; kiseonik, potrošnja.

Introduction

Hearth failure (HF) is a complex clinical condition caused by spectrum of various heart diseases. It is a leading cause of morbidity and mortality among people over 65 years of age, with a current prevalence of more than 23 million cases and rising incidence worldwide [1, 2].

Left ventricular ejection fraction (LVEF) is reduced in more than a half of patients with HF [3]. The cardiopulmonary exercise testing (CPET) has an important role in the assessment of HF status [4], especially because the wealth of previous investigations has consistently demonstrated its prognostic values in the HF population [4–8]. The two most frequently assessed variables obtained from CPET are the peak oxygen consumption (VO_2) and the minute ventilation-carbon dioxide production (VE/VCO_2) relationship. VO_2 was the first CPET variable used in clinical practice, and now it is considered to be the diagnostic and prognostic gold standard in HF [9]. Furthermore, previous investigations reported that VE/VCO_2 slope was also significant predictor of survival of patients with HF as the continuous variable and even more prognostically superior to peak VO_2 [6, 10, 11]. These variables, along with LVEF, represent the pivotal predictor of morbidity and mortality in the patients with HF [12, 13]. However, correlation between LVEF and exercise capacity remains unclear, and still needs to be elucidated [14, 15].

Two-dimensional (2D) and three-dimensional (3D) echocardiography represent the most commonly performed noninvasive cardiac imaging tests used to quantitatively assess cardiac volumes, the LVEF, stroke volume, and cardiac output in the patients with HF [16, 17]. 3D speckle-tracking echocardiography (3D-STE) is a new promising method for the quantitative assessment of LV volumes, myocardial strain and strain rate in longitudinal, radial and circumferential dimension [18]. Moreover, 3D-STE overcomes the usual drawbacks of conventional 2D echocardiography, such as the modest interobserver, intraobserver, and the test-retest reproducibility of specific structural and functional parameters [19].

Therefore, this study aimed to explore the relationship between the exercise capacity parameters (VO_2, VE/VCO_2) and 3D-STE imaging of LV function in the HF patients with reduced LVEF.

Methods

This cross-sectional study was conducted at the Department of Cardiology, University Hospital "Dr Dragiša Mišović" Belgrade, Serbia from February 2012 to February 2016. The study was approved by the local Ethics Committee. Informed consent was obtained from all participants after all procedures had been fully explained to them and prior to the clinical and laboratory examinations.

The present study included 80 consecutive patients, with diagnosed ischemic left ventricular systolic dysfunction (LVEF < 45%) and sinus rhythm [3], referred to our clinic due to the condition evaluation. The patients with age over 75 years, severe angina syndrome, atrial fibrillation, severe valvular disease, anemia or chronic obstructive pulmonary disease were excluded from the study.

All patients underwent a physical examination, including anthropometric measures (height, weight), laboratory testing [creatinine, the fasting glucose level, glycated hemoglobin (HbA1c), total cholesterol, high and low density lipoprotein (HDL and LDL) cholesterol, triglycerides, C-reactive protein (CRP), N-terminal *pro* brain natriuretic peptide (NT-*pro* BNP)), echocardiography, and CPET]. Additionally, the body mass index (BMI) was calculated for each patient. Based on the proposed values of analyzed CPET variables (VO_2, VE/VCO_2) [8, 20], all participants were further subdivided into 4 subgroups: VO_2 peak $\leq$ 15 mL/kg/min, VO_2 peak > 15 mL/kg/min, VE/VCO_2 slope < 36 and VE/VCO_2 slope $\geq$ 36.

Echocardiography

The echocardiographic examinations were performed by the commercially available Vivid 7 (GE Vingmed, Horten, Norway) ultrasound machine equipped with a 2.5 MHz transducer with harmonic capability. All echocardiographic data were analyzed off-line.

Standard 2D echocardiographic examination

The 2D echocardiographic parameters were obtained as the average value of three consecutive cardiac cycles. The left atrial (LA), left ventricular end-systolic (LVESD) and end-diastolic (LVEDD) diameters, the left ventricular end-systolic (LVESV) and end-diastolic (LVEDV) volumes, the left ventricular posterior wall thickness (PWT), septum thickness, and right ventricle systolic pressure (RVSP) were determined according to the current recommendations [21]. LVEF was estimated by using the biplane method. Transmitral Doppler inflow and tissue pulsed Doppler were obtained in the view of the four chamber apex. The pulsed Doppler measurements included the transmitral early diastolic peak flow velocity (E), late diastolic flow velocity (A), E/A ratio, E velocity deceleration time (DT) and ratio between mitral flow E peak velocity and tissue Doppler derived e' (E/e' ratio) of the septal mitral annulus (MVEes) [22]. The tissue Doppler imaging was used to obtain the left ventricular myocardial velocities in the apical four-chamber view.

2D echocardiography left ventricle strain

The 2D longitudinal strain was performed by Automated Functional Imaging (AFI). The algorithm tracked the wall motion and calculated the percentage of lengthening or shortening in a set of three longitudinal 2D-image planes (apical long, 2-chamber and 4-chamber) and displayed the results for each plane. It then combined the results of all three planes in a single summary, which presented the analysis for each segment along with a global peak strain value for the left ventricle [18]. The frame rate ranged between 50 and 70 Hz.

3D echocardiography examination

A full-volume acquisition of the left ventricle, which required the further analysis, was obtained by harmonic imaging from an apical approach. Six electrocardiogram-gated consecutive beats were acquired during the end-expiratory breath-hold (6–8 s) to generate full volume. The frame rate was higher than 30 frames/s.

All data sets were stored digitally and analyzed off-line by a commercially available software 4D Auto LVQ software (EchoPAC 110.1.2; GE-Healthcare). The software automatically identified in 3D endocardial border of the left ventricular cavity and provided the left ventricular volumes, cardiac output (CO), stroke volume (SV), EF, and left ventricular sphericity index. After that, an automatic trace of the epicardial border was displayed to detect the region of interest required for the 3D assessment of myocardial deformation parameters (speckle tracking). The 3D deformation parameters, global longitudinal strain (GLS), global circumferential strain (GCS), global radial strain (GRS), and global area strain (GAS), were calculated as the weighted averages of the regional values from the 17 myocardial segments at end-systole [23]. If three, or more segments were rejected, the global strain values were not calculated, and these patients were excluded.

Cardiopulmonary exercise testing (CPET)

All patients underwent a maximum symptom-limited (fatigue and/or dyspnea) treadmill exercise test according to the modified Noughton protocol [24]. It consisted of 6 levels lasting for 3 minutes, with constant seed of 3 km/h, and start 0 elevation increasing for 3.5% for each interval. The patients were encouraged to continue with the test as long as their respiratory exchange ratio exceeded 1. The peak oxygen uptake (VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE) were assessed with the breath-by-breath gas analysis (CARDIOVIT CS-200 Ergo-Spiro system; Schiller AG, Baar, Switzerland). Spirometry was done in all participants before the cardiopulmonary exercise testing, including forced expiratory volume in the first second (FEV_1) and the measurement of forced vital capacity (FVC), which was computed as a percentage of predicted values, considering age and gender. VO_2 was defined as an average value within the last 20 seconds of exercise and expressed as mL/kg/min and METs (1 MET equals 3.5 mL of oxygen uptake per kilogram of body weight per minute). The ventilatory anaerobic threshold and oxygen uptake at this level, expressed as the percentage of VO_2, was determined in all the participants. The VE/VCO_2 slope, which showed the linear increase of ventilation relative to the carbon dioxide production, was computed automatically by the Schiller computer system.

Statistical analysis

The statistical analyses were performed using the IBM SPSS Statistics for Windows Software (Version 20.0, IBM Corp, Armonk, NY, USA) and R: A Language and Environment for Statistical Computing (Version 3.0.3, R Foundation for Statistical Computing, Vienna, Austria). The results were presented as counts (percentage) or mean ± standard deviation. The group comparisons were performed using the Student's *t*-test or Mann-Whitney U-test. The correlation between two numerical variables was tested using the Pearson correlation analysis. The χ^2 analysis was conducted to assess a statistical significance between categorical data. The receiver operating characteristic (ROC) analysis was performed to determine the best parameter of 3D echocardiography in different subgroups and to calculate the area under the curve, cut-off value, sensitivity, specificity, positive likelihood ratio (LR +), and negative LR (LR-) for the investigated parameters.

Results

The demographic and clinical parameters of the study population are presented in Table 1. The participants were of similar age and gender distribution without significant differences between examined subgroups.

The level of CRP was significantly higher in the subgroup of subjects with a peak VO2 less or equal to 15 mL O2/kg per min compared to the subgroup of subjects with a peak VO2 greater than 15 mL O2/kg per min ($p = 0.028$) (Table 1). Furthermore, the levels of CPR, NT-pro BNP and creatinine were significantly higher in the subgroup of subjects with a VE/VCO2 slope greater or equal to 36 compared to the subgroup of subjects with a VE/VCO_2 slope less than 36 ($p = 0.001$; $p = 0.002$; $p = 0.019$ respectively) (Table 1). The levels of other analyzed clinical parameters were similar and without significant differences between the investigated subgroups.

The parameters of 2DE in the investigated subgroups are presented in Table 2. RVSP was significantly increased in in the subgroups of subjects with a peak VO_2 less or equal to 15 mL O_2/kg per min compared to the subgroups of subjects with a peak VO_2 greater than 15 mL O_2/kg per min ($p = 0.005$). Additionally, RVSP was significantly increased ($p = 0.003$, respectively) while EF biplane, MVEes, and GLS were significantly decreased ($p = 0.006$; $p = 0.036$; $p = 0.029$, respectively) in the subgroups of subjects with a VE/VCO_2 slope greater or equal to 36 compared to the subgroups of subjects with a VE/VCO_2 slope less than 36 ($p = 0.019$; $p = 0.003$, respectively) (Table 2).

The 3DE LV strain analysis revealed that the global longitudinal, circumferential, radial and area strains were significantly lower in the subgroups of subjects with a peak VO_2 less or equal to 15 mL O_2/kg per min and with a VE/VCO_2 slope greater or equal to 36 compared to the subgroups of subjects with a peak VO_2 greater than 15 mL O_2/kg per min ($p = 0.014$; $p = 0.037$; $p = 0.003$; $p = 0.010$, respectively) and with a VE/VCO_2 slope less than 36 ($p = 0.005$; $p = 0.038$; $p = 0.009$; $p = 0.009$, respectively). The same trend was observed for the 3D EF (Table 3).

Table 1

Demographic characteristics and clinical parameters of study population

Variables	VO_2 (mL/kg/min)		*p* value	VE/VCO_2 (slope)		*p* value
	≤ 15 (n = 25)	> 15 (n = 55)		< 36 (n = 60)	≥ 36 (n = 20)	
Age (years)	64.9 ± 7.5	63.3 ± 8.9	0.424 [a]	63.4 ± 9.2	64.6 ± 7.7	0.597 [a]
Gender (M/F)	18/7	35/20	0.348 [b]	45/15	13/7	0.466 [b]
BMI (kg/m^2)	27.2 ± 2.9	26.8 ± 3.4	0.577 [a]	27.1±3.2	26.5 ± 3.4	0.490 [a]
NT-pro BNP (pg/mL)	1340.4 ± 1842.7 (311; 186–2073)	1146.8 ± 2775.4 (308; 121–709)	0.352 [c]	789.3 ± 2303 (241; 99–656)	2221.5 ± 2697.2 (777; 300–3723)	0.002 [c]
Creatinine (umol/L)	101.8 ± 50.4	98.3 ± 49.9	0.715 [c]	91.3 ± 40.7	119 ± 63.5	0.019 [c]
Fasting plasma glucose (mmol/L)	7.6 ± 3.3	6.4 ± 2.2	0.080 [a]	6.1 ± 1	7.8 ± 3.5	0.053 [a]
HbA1c (%)	6.6 ± 1.5	6.2 ± 1.1	0.120 [a]	6.1 ± 1	6.8 ± 1.7	0.089 [a]
Cholesterol (mmol/L)	4.8 ± 1.2	5 ± 1.2	0.337 [a]	5.1 ± 1.3	4.5 ± 1	0.120 [a]
LDL (mmol/L)	3 ± 1	3 ± 1.1	0.829 [c]	3.1 ± 1.1	2.7 ± 0.8	0.074 [c]
HDL (mmol/L)	1.2 ± 0.4	1.2 ± 0.4	0.603 [a]	1.2 ± 0.4	1.2 ± 0.4	0.536 [a]
Triglycerides (mmol/L)	1.7 ± 1	1.5 ± 0.8	0.485 [c]	1.7 ± 1	1.4 ± 0.85	0.067 [c]
CRP (mg/L)	4.2 ± 7 (3.3; 1.9–7.9)	6 ± 7 (2.1; 1.4–3.7)	0.028 [c]	3.2 ± 4.3 (2; 1.3–3.7)	8.6 ± 10.2 (3.8; 2.6-8)	0.001 [c]

Data are presented as mean ± standard deviation or number of participants; for parameters NT-pro BNP and CRP median, 25 and 75 percentile were also presented.
M – male; F – female; BMI – body mass index; NT-pro BNP – N-terminal pro brain-type natriuretic peptide; LDL – low-density lipoprotein; HDL – high-density lipoprotein; CRP – C reactive protein; VO2 – peak oxygen consumption; VE/VCO2 – ventilation/carbon dioxide production relationship. [a]Student's *t*-test; [b]χ^2 test; [c]Mann-Whitney U-test.

Table 2

Two-dimensional echocardiography parameters in the investigated group

Variables	VO_2 (mL/kg/min)		*p* value	VE/VCO_2 (slope)		*p* value
	≤ 15 n = 25	> 15 n = 55		< 36 n = 60	≥ 36 n = 20	
LA (mm)	45.2 ± 6.5	43.7 ± 6.3	0.343 [a]	43.5 ± 6	46 ± 7.2	0.119 [a]
LVEDD (mm)	58.9 ± 8.5	55.7 ± 7.5	0.114 [a]	55.7 ± 7.8	58.9 ± 7.8	0.107 [a]
LVESD (mm)	48.1 ± 7.9	45.8 ± 7.9	0.214 [a]	45.7 ± 8	48.4 ± 7.6	0.171 [a]
LVEDV/BSA (mL/m^2)	78 ± 22	77.1 ± 26.5	0.884 [a]	74.6 ± 26.2	84.4 ± 20.9	0.115 [a]
LVESV/BSA (mL/m^2)	51.5 ± 20.1	49.3 ± 23.1	0.678 [a]	47.4 ± 23.1	56.5 ± 18.3	0.097 [a]
EF biplane (%)	34.1 ± 8.1	37.2 ± 6.3	0.200 [b]	37.6 ± 6.6	32.3 ± 7.1	0.006 [b]
RVSP (mmHg)	41.4 ± 19.9	30.6 ± 10.1	0.005 [a]	31.2 ± 12.4	40.9 ± 13.8	0.003 [a]
MVEes	0.047 ± 0.013	0.050 ± 0.015	0.492 [a]	0.051 ± 0.015	0.044 ± 0.011	0.036 [a]
GLS (%)	10.7 ± 3.7	12.2 ± 3.3	0.084 [b]	12.3 ± 3.2	10.4 ± 3.8	0.029 [b]

Data are presented as mean ± standard deviation.
LA – left atrium; LVEDD – left ventricular end diastolic diameter; LVED – left ventricular end systolic diameter; LVEDV – left ventricular end diastolic volume; LVESV – left ventricular end systolic volume; EF biplane-two-dimensional ejection fraction; BSA – body surface area; RVSP – right ventricle systolic pressure; MVEes-ratio between mitral flow E peak velocity and tissue Doppler derived e' of the septal mitral annulus; GLS – global longitudinal strain; VO_2 – peak oxygen consumption; VE/VCO_2 – ventilation/carbon dioxide production relationship.
[a]Student's *t*-test; [b]Mann-Whitney U-test.

Table 3

Three – dimensional ejection fraction and variables

Variables	VO_2 (mL/kg/min)		*p*-value	VE/VCO_2 (slope)		*p*-value
	≤ 15 (n = 25)	> 15 (n = 55)		< 36 (n = 60)	≥ 36 (n = 20)	
3D EF (%)	33.1 ± 8.9	37.7 ± 6.7	0.011 [a]	38.2 ± 6.6	31.6 ± 8.4	0.001 [a]
Longitudinal strain (%)	8.1 ± 3.3	10.3 ± 3.8	0.014 [a]	10.4 ± 3.5	7.8 ± 3.7	0.005 [a]
Circumferential strain (%)	7.7 ± 3	9.6 ± 3.9	0.037 [a]	9.5 ± 3.7	7.7 ± 3.3	0.038 [a]
Radial strain (%)	18.4 ± 7.9	24.9 ± 10.3	0.003 [a]	24.7 ± 9.8	18.3 ± 9.3	0.009 [a]
Area strain (%)	14.1 ± 5.2	18 ± 6.3	0.010 [a]	17.9 ± 6	13.9 ± 6	0.009 [a]

Data are presented as mean ± standard deviation; 3D EF – three-dimensional ejection fraction; VO_2 – peak oxygen consumption; VE/VCO_2 – ventilation/carbon dioxide production relationship; [a] Student's *t*-test.

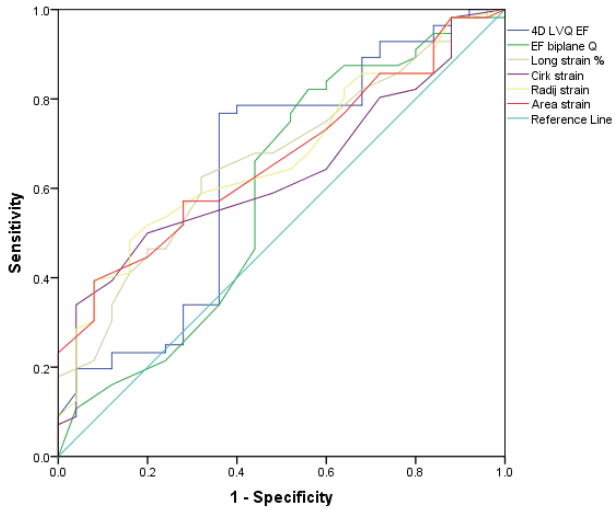

Fig. 1 – Receiving operating characteristic (ROC) curve of the investigated parameters in relation to a peak oxygen consumption (VO_2) values.

An impact of a peak VO_2 and VE/VCO_2 slope values on the 3DE parameters was further investigated by the ROC analysis. The ROC curve areas for the 3DE parameters in relation to a peak VO_2 values were presented in Figure 1 and Table 4. The highest area under the ROC curve was observed for the radial strain 0.67 [95% confidence interval (CI) 0.55–0.79] (p = 0.015). When considering the highest level of sensitivity, the cut-off value for radial strain was 24.5 %; with the sensitivity of 84 % and specificity of 48 %, while the positive and negative likelihood ratios (LR+, LR-) were 1.62 and 0.33, respectively.

The ROC curve areas for the 3DE parameters in relation to the VE/VCO_2 slope values were presented in Figure 2 and Table 5.

The highest area under the ROC curve was observed for the 3D EF 0.73 [95% confidence interval (CI) 0.60–0.86] (p = 0.001). When considering the highest level of sensitivity the cut-off value for 3D EF was 36.04 %; with the sensitivity of 78.3 % and specificity of 75.9 %, while LR+, LR- were 3.24 and 0.29, respectively.

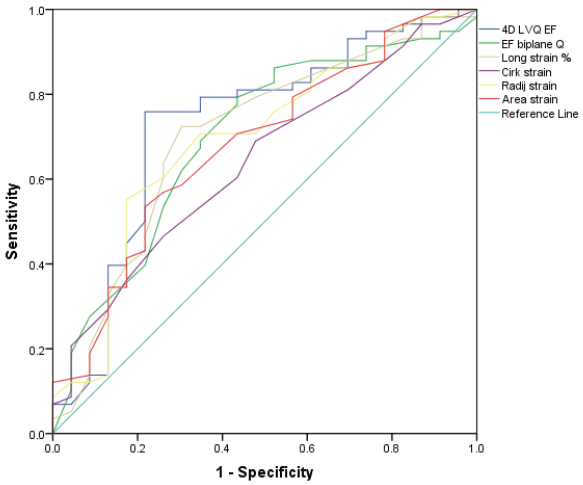

Fig. 2 – Receiving operating characteristic (ROC) curve of the investigated parameters in relation to a ventilation/carbon dioxide production relationship (VE/VCO_2) slope values.

We observed a significantly positive correlation between the peak VO_2 values and parameters of 3DE (Table 6). On the other hand, there was also a significantly negative correlation between the VE/VCO_2 slope values and parameters of 3DE (Table 6).

Table 4

Receiving operating characteristics (ROC) curve of the investigated parameters in relation to a peak oxygen consumption (VO_2) values

Variables	Area	SE	95% CI		p value
			lower bound	upper bound	
EF biplane (%)	0.589	0.071	0.444	0.735	0.201
3D EF (%)	0.641	0.071	0.502	0.781	0.043
Longitudinal strain (%)	0.667	0.062	0.545	0.789	0.017
Circumferential strain (%)	0.634	0.063	0.511	0.757	0.055
Radial strain (%)	0.670	0.061	0.550	0.790	0.015
Area strain (%)	0.667	0.061	0.548	0.786	0.017

EF – ejection fraction; 3D EF – three dimensional EF; SE – standard error; CI – confidence interval.

Table 5

Receiving operating characteristics (ROC) curve of the investigated parameters in relation to a VE/VCO_2 slope values

Variables	Area	SE	95% CI		p value
			lower bound	upper bound	
EF biplane (%)	0.698	0.066	0.569	0.827	0.006
3D EF (%)	0.729	0.067	0.598	0.861	0.001
Longitudinal strain (%)	0.702	0.067	0.571	0.833	0.005
Circumferential strain (%)	0.640	0.066	0.510	0.770	0.050
Radial strain (%)	0.692	0.067	0.562	0.822	0.007
Area strain (%)	0.680	0.066	0.551	0.809	0.012

EF – ejection fraction; 3D EF – three dimensional EF; VE/VCO_2 – ventilation/carbon dioxide production relationship. SE – standard error; CI – confidence interval.

Table 6
Correlation between the parameters of cardiopulmonary exercise testing and three-dimensional echocardiography parameters

Variables	VO_2 (mL/kg/min)	VE/VCO_2 (slope)
3D EF (%)	0.258 *	-0.358 **
Longitudinal strain (%)	0.368 **	-0.343 **
Circular strain (%)	0.317 **	-0.316 **
Radial strain (%)	0.376 **	-0.366 **
Area strain (%)	0.373 **	-0.339 **

EF – ejection fraction; 3D EF – three dimensional EF; VE/VCO_2 – ventilation/carbon dioxide production relationship; VO_2 – peak oxygen consumption.
*** $p < 0.05$; ** $p < 0.001$.**

Discussion

HF prevalence ranges between 2% and 3% in the general population [25], including the Serbian population [26], with increasing trend due to the population ageing. LVEF is an established predictor of adverse cardiovascular outcomes in the HF patients [3]. However, several studies suggested that its prognostic utility of HF was limited due to the poor sensitivity in detecting early myocardial dysfunction [27, 28]. Echocardiography remains the most commonly performed noninvasive cardiac imaging test for the patients with HF in routine clinical practice [16, 17]. Its capacity to quantify the complex cardiac structures and provide insights into the myocardial functions and mechanics has dramatically improved with development of 3D-STE, as the novel method for the quantitative assessment of LV volumes, myocardial strain and strain rate in longitudinal, radial and circumferential dimension [18].

A recent meta-analysis conducted by Ma et al. [17] investigated clinical utility of 3D-STE for the LV function in the patients with chronic HF. The authors included 7 case-control studies with a total of 375 patients with HF and 181 healthy control participants in the final review. The meta-analysis results showed that the LVEF in the HF patients was significantly lower than in the controls. Furthermore, global longitudinal, circumferential and radial strain were also impaired in the HF patients compared to the controls. Based on the provided results they concluded that the LV function in the patients with HF can be noninvasively and objectively measured by 3D-STE [17] which is in agreement with our results. Several previous studies, not included in this meta-analysis, also investigated the utility of different strain and strain rates assessed by 3D-STE in the HF patients [29, 30]. Kleijn et al. [29] stated that the area strain represented the echocardiographic standard for the quantitative assessment of global and regional LV function. On the other hand, Zhang et al. [30] reported that the longitudinal, circumferential and radial strains were significantly associated with a prognosis in chronic systolic HF which was in accordance with our results. We demonstrated the significantly lower values of analyzed strains and 3D EF in both subgroups with the poor prognosis of HF (VO_2 peak $\leq$ 15 mL/kg/min, VE/VCO_2 slope $\geq$ 36). Furthermore, we demonstrated that the highest areas under the ROC curves were for radial strain in relation to a peak VO_2 values and 3D EF in relation to a VE/VCO_2 slope values. Our results are also in agreement with the study of Cho et al. [31] who proposed a multicriteria echocardiographic analysis and stated that the clinical approach needed to be multiparametric, as the sum of different positive parameters permitted an improved patient risk diagnosis. Additionally, our results of 2DE are in accordance with the previously reported results related to the patients with HF [32].

Interestingly, a recent EuroHeart Failure survey showed that 85% of patients suffering from HF underwent the echocardiography testing, while only 4.4% underwent the cardiopulmonary exercise test (CPET) [33]. However, in our clinical center, the CPET is widely used in the clinical assessment of patients with HF and the main objective of this study was to explore the relationship between the exercise capacity parameters (VO_2, VE/VCO_2) and 3D-STE imaging of LV function in the HF patients with reduced LVEF. We observed a significantly positive correlation between the peak VO_2 values and parameters of 3DE, and a significantly negative correlation between the VE/VCO_2 slope values and the parameters of 3DE which is in agreement with the previously reported results [34, 35]. Namely, Peterson et al. [34] stated that the 3D-STE measures had a strong linear association with estimates of functional capacity. Additionally, Donal et al. [35] reported a moderate correlation between 3D-STE and the functional capacity parameters.

The prognostic value of exercise testing is well-established in the assessment of HF status [4]. A peak VO_2 and VE/VCO_2 slope are the most frequently assessed variables obtained from CPET and previous studies confirmed their utility in the assessment of patients with HF [4-8]. However, it is important to emphasize that it was reported that VE/VCO_2 slope may be a better predictor of outcome than peak VO_2 in the HF population [5, 6]. There are two possible reasons for the differences between the prognostic value of the VE/VCO_2 slope and peak VO_2. Potential weaknesses of peak VO_2 are its dependence on the subject effort and the influence of peripheral metabolism. On the other hand, unlike peak VO_2, the VE/VCO_2 slope is generally linear and remains relatively constant throughout a progressive exercise test that makes it independent of subject effort. Therefore, in the event of submaximal effort, the VE/VCO_2 slope would theoretically maintain the diagnostic and prognostic significance [5, 6].

The analysis of clinical parameters revealed that the levels of CRP were significantly increased in the HF patients in the subgroups with a peak VO2 less or equal to 15 mL O2/kg per min and with a VE/VCO2 slope greater or equal to 36. These results suggest that the patient with a poor prognosis of HF are characterized with the increased inflammation. It was stated that the increased CRP levels in the patients with HF may be a consequence of an ischemic necrosis that initiate this potent inflammatory stimulus [36]. Our results are in accordance with the study of Liu et al. [37] that reported a positive correlation between the increased CRP level and the increased level of serum complement factors C3, C4, C5b9 in the HF patients.

Furthermore, we observed the significantly higher values of creatinine in the HF patients with a peak VO_2 less or

equal to 15 mL O_2/kg per min and with a VE/VCO_2 slope greater or equal to 36 compared to the HF patients with a peak VO_2 greater than 15 mL O_2/kg per min and with a VE/VCO_2 slope less than 36. These results imply worsening of renal function in the patients with HF. There is increasing evidence that persistent increase in creatinine is correlated with a poor prognosis of patients with HF [38–42], which is in agreement with our results.

HF is characterized by the dysfunctional natriuretic peptide system. Tsutamoto et al. [43] were the first to demonstrate that a single BNP measurement was predictable of mortality in HF. Moreover, Koglin et al. [44] reported that the patients with chronic HF and high BNP levels had a higher probability of deterioration of their functional status or death than those with only moderate increased BNP levels. In our study, the levels of NT-pro BNP were significantly higher in the HF patients with poor prognosis. A wealth of previous studies also reported that the plasma concentrations of NT-pro BNP are increased in the patients with HF and accurately predict LVEF as well as morbidity and mortality in these patients [45–47] which is in line with our results.

Conclusion

The results of this study provide further evidence that LV function can be noninvasively and objectively measured by 3D-STE. The observed significant correlation between examined parameters suggests that LVEF and strain derived by 3D echocardiography are associated with exercise capacity in the patients with HF.

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The effects of the physical procedures in patients with diabetic neuropathy

Efekti fizikalne terapije kod bolesnika sa dijabetesnom neuropatijom

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Abstract

Background/Aim. Neuropathy represents the most frequent complication in the patients with diabetes mellitus (DM). Symmetric distal sensorimotor polyneuropathy (DSP), which represents the most frequent type of diabetic neuropathy, is present in 30% of hospitalized diabetic patients. The aim of our study was to compare the effects of physical therapy (PT) and alpha-lipoic acid (ALA) supplementation on pain reduction and quality of life improvement in the DSP patients. **Methods.** The study was performed on 60 adult patients with DM type 2 and DSP. The patients were randomly divided into 2 groups: group A (n = 30) was treated by PT and group B (n = 30) was treated by ALA. The study lasted 6 months during which 3 diagnostic-therapeutic cycles were performed. To asses their pain before and after every of 3 cycles, we used visual analog scale (VAS). We also evaluated quality of life before the 1st and after the 3rd cycle with the European Quality of Life Questionnaire (EQ-5D-3L). To analyze results between groups we used mixed between-within subjects ANOVA and statistical significance was set on $p < 0.05$. **Results.** Pain intensity showed statistically significant influence of both PT and ALA ($\lambda = 0.028$; $p < 0.001$). A statistically significant difference between the effects of those two therapy modalities was observed ($F = 4.78$; $p < 0.05$): PT reduced pain to the greater extent than ALA. A statistically significant improvement was found in the domain of pain/discomfort both in the group A ($\eta = 0.54$, $p < 0.001$) and group B ($\eta = 0.57$, $p < 0.001$), as well as anxiety/depression (group A: $\eta = 0.32$, $p = 0.008$; group B: $\eta = 0.22$, $p < 0.019$) and EQ-VAS (both groups, $p < 0.05$). **Conclusion.** Our research showed that physical therapy had a greater influence in pain reduction than alpha-lipoic acid in the patients with DSP.

Key words:
diabetic neuropathies; physical therapy modelaties; treatment outcome.

Apstrakt

Uvod/Cilj. Neuropatija predstavlja najčešću komplikaciju kod bolesnika sa dijabetes melitusom (DM). Simetrična distalna senzomotorna polineuropatija (DSP) predstavlja najčešći tip dijabetesne neuropatije i zastupljena je kod 30% hospitalizovanih bolesnika sa dijabetesom. Cilj naše studije je bilo poređenje efekata primenjene fizikalne terapije (PT) i alfalipoične kiseline (ALA) na smanjenje bola i kvalitet života kod bolesnika sa DSP. **Metode.** U studiju je bilo uključeno 60 odraslih bolesnika sa DM tip II i DSP. Bolesnici su slučajnim izborom bili podeljeni u 2 grupe: Grupa A (n = 30) je bila tretirana PT, a grupa B (n = 30) primenom ALA. Tokom studije, koja je trajala šest meseci, sprovedena su tri dijagnostičko-terapijska ciklusa. Za procenu bola pre i posle svakog od tri ciklusa korišćena je vizuelno analogna skala (VAS). Takođe, evaluiran je kvalitet života pomoću Evropskog upitnika o kvalitetu života (EQ-5D-3L). Za poređenje rezultata među grupama korišćena je kombinovana analiza varijanse, a značajnost razlike prihvaćena je na nivou $p < 0,05$. **Rezultati.** Intenzitet bola bio je statistički značajno smanjen kod obe grupe ispitanika primenom PT i ALA ($\lambda = 0,028$; $p < 0,001$). Utvrđena je statistički značajna razlika između dve vrste primenjenih terapija ($F = 4,78$; $p < 0,05$): PT je dovela do značajnije redukcije bola od ALA. Došlo je do statistički značajnog poboljšanja kvaliteta života u domenu bol/diskomfor u grupi A ($\eta = 0,54$, $p < 0,001$) i grupi B ($\eta = 0,57$, $p < 0.001$),

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u domenu ankioznost/depresivnost (grupa A: $\eta = 0{,}32$, $p = 0{,}008$; grupa B: $\eta = 0{,}22$, $p < 0.019$) i domenu EQ-VAS (obe grupe, $p < 0.05$). **Zaključak.** Rezultati studije pokazuju da primena fizikalne terapije ima veći uticaj na smanjenje bola u poređenju sa primenom alfalipoične kiseline kod bolesnika sa DSP.

Ključne reči:
dijabetičke neuropatije; fizikalna terapija, metodi; tioktinska kiselina; lečenje, ishod.

Introduction

Neuropathy represents the most frequent complication in the patients with diabetes mellitus (DM) [1]. Symmetric distal sensorimotor polyneuropathy (DSP), which represents the most frequent type of diabetic neuropathy, is present in 30% of hospitalized diabetic patients [2]. Prevalence of symmetrical DSP in the whole population of diabetics is 13%–68% [3].

DSP is characterized by persistent or periodical pain localized mainly in feet, which is provoked even by light touch, gets worse during the night, but reduced during walking. This pain is very unpleasant and persistent, lasts for years, its intensity and frequency disturbs sleep and rest and reduces work energy and thus impairs the overall quality of life, contributing to a loss of autonomy and independence in performing many daily activities and the reduction of working capacity. Thus, the pain reduction and improvement of quality of life represent the challenge of modern medicine [4].

Often, the primary objective of a therapy is to protect the lower extremities from damage caused by the loss of protective sensibility as well as the reduction of pain, which improves physical well-being and quality of life of DSP patients [5]. The DSP therapy is complex, directed towards both causal treatment (achievement and maintenance of an adequate level of glucoregulation, liporegulation and regulation of arterial blood pressure as well as pharmacological treatment) and symptomatic treatment. The symptomatic treatment includes antidepressants, anticonvulsants, opioids and local anesthetics as well as the physical therapy. The therapy directed towards the pathogenetic process involves aldose reductase inhibitors, alpha-lipoic acid (ALA), benfotiamine, protein kinase C inhibitors, gene therapy, gama-linoleic acid, immunotherapy, and others [6].

ALA is an endogenous antioxidant which gained a scientific support as the medicament of choice in diabetic neuropathy treatment [7, 8]. It is particularly important to emphasize the necessity of timely implementation of the therapy before the occurrence of severe and irreversible changes of the nerves. The application of physical therapy (PT) in the treatment of patients with DSP is becoming increasingly important, especially as the analgesic therapy. The most frequently applied physical agents are transcutaneous electrical nerve stimulation, pulsed magnetic field therapy, stable galvanization, exercise therapy, etc. By reducing pain, the physical agents improve quality of life of patients.

Available scientific literature contains no research paper that compares the effects of combined PT vs ALA supplementation on the patient-centered treatment endpoints in diabetics with DSP. Thus, the aim of our research was to compare the effects of PT and ALA supplementation on pain reduction and quality of life improvement in DSP patients.

Methods

This clinical open prospective randomized intervention study was performed in the Center for Physical Medicine and Rehabilitation of the Clinical Centre Kragujevac. The study was conducted according to the principles of Helsinki Declaration and it was approved by the Independent Ethics Committee of Clinical Centre "Kragujevac", Kragujevac, Serbia.

The study was performed on 60 adult patients with DM type 2 and DSP. The informed consent was obtained from the patients. The inclusion criteria were the following: 1) DSP presence for more than 2 months, diagnosed by the electromioneurographic findings and the presence of symptoms and signs of DSP (pain, paresthesia, hyperesthesia to anesthesia, muscle weakness); 2) antidiabetic therapy constant for previous 6 months; 3) written informed signed consent of the patient. Exclusion criteria included: 1) a number of diseases and conditions [vitamin B12 deficiency, alcoholism, chronic renal insufficiency, thyroid dysfunction, immunodeficient diseases, systemic connective tissue disease, severe liver damage, cerebrovascular ischemia, cardiac decompensation, acute coronary syndrome within the previous 6 months, uncontrolled elevated blood pressure (systolic pressure >160 mmHg or diastolic pressure > 80 mmHg), subjection to chemotherapy in the past 10 years, and states after severe polytrauma]; 2) usage of drugs that can cause damage to the peripheral nerves (vincristine, cis-platinum, paclitaxel, streptomycin, isoniazid, ethionamide, dapsone, nitrofurantoin, metronidazole, misonidazole, emetine, chloroquine, amiodarone, carbamazepine, phenytoin, hydralazine, indomethacin), 3) the existence of any contraindication for the application of physical agents planned for the use in the study (pregnancy, fever, cancer, acute infectious disease, decompensation of the vital organs, presence of metals in the tissue, a disease or damage to the integrity of the skin at the site of electrode application). For the application of ALA, the exclusion criteria were hypersensitivity to the active substance of the drug, or any other ingredients.

By using the Microsoft Excel Rand between function, the patients were randomly divided into 2 groups: 1) group A (n = 30), DM DSP patients who were treated by PT, and 2) group B (n = 30), DM DSP patients who were treated by ALA.

The study lasted 6 months, during which 3 diagnostic-therapeutic cycles were performed. Each diagnostic-therapeutic cycle lasted 14 days (2 weeks), while pause between them lasted 6–7 weeks.

Combined PT included: 1) transcutaneous electrical nerve stimulation (TENS), 2) pulsed electromagnetic field therapy (PEMF), 3) stable galvanization (SG) and 4) exercise. TENS was applied once daily for 30 minutes, using TENS-2 (Electronic Design Medical, Serbia) apparatus on both legs, longitudinally (frequency: 85 Hz, impulse duration: 4 ms). PEMF was applied once daily for 30 minutes, on Magomil-2 apparatus (Electronic Design Medical, Serbia), along both lower legs and feet, over the antenna (frequency: 10 Hz, intensity: 40 mT). SG was applied once daily for 20 minutes, on Galvan Plus apparatus (Electronic Design Medical, Serbia), using standard rectangular electrodes placed longitudinally descendently along both legs (intensity 0.1–0.5 mA/cm^2, according to the subjective experience of patients). Exercise was applied once daily for 30 minutes, according to individually adapted program (active-assisted and active exercises for strengthening muscles of legs and improvement of range of motion in all joints of legs).

The ALA supplementation (Thiogamma®, Wörwag Pharma, Germany) was performed according to the manufacturer's instructions (indications, dosing, precautions, etc.) and standard clinical practice. From 2nd to 15th day of hospitalization, the patients were treated with the intravenous application of preparation of alpha-lipoic acid (600 mg in 500 ml 0.9% NaCl). Upon completion of hospitalization, and throughout the entire study period, those participants continued to regularly take ALA orally in dose of 600 mg (one tablet per day, in the morning, before breakfast).

At admission to hospital (before every diagnostic-therapeutic cycle) as well as after every diagnostic-therapeutic cycle, the patients were asked to assess their pain level by using 100 mm visual analog scale (VAS score). To asses their quality of life, the patients were asked to fulfill the European Quality of Life Questionnaire (EQ-5D-3L) before the 1st diagnostic-therapeutic cycle, and after the 3rd (the last) diagnostic-therapeutic cycle. EQ-5D-3L is a general standardized indicator of quality of life that assesses 5 domains. These 5 domains provide an assessment within 3 levels ranging from 1 (best quality of life) to 3 (worst quality of life): 1) mobility, 2) self-care, 3) perform in regular daily activities, 4) pain/discomfort and 5) anxiety/depression. EQ-5D also includes the 20 cm vertical visual analog scale (EQ-VAS), so called the assessment scale, where the respondent assesses the quality of their own overall health status scores of 0 (worst) to 100 (best) [9, 10].

The statistical analysis was performed in the SPSS 20.0. The continuous variables are presented as mean ± standard deviation (SD), while the categorical variables are presented as proportions, i.e., percentage of an individual category. The normality of data distribution of all examined continuous variables was examined using the Shapiro-Wilk test. For comparison of mean values of continuous variables within the tested groups at the beginning and the end of the study *t*-test (for related samples with normal distribution) and Wilcoxon's matched pairs test (for the outcomes that do not follow a normal distribution) were used. In order to compare the mean values of continuous variables between the groups, the independent *t*-test or Mann-Whitney *U* test was used, depending on whether the distribution was, or was not normal. The Chi-square (χ^2) test was used to determine the significance of differences in frequencies of certain categories in the categorical variables, and the Fisher's test when the frequency of some categories was small. Testing the efficacy of two therapeutic modalities to reduce pain in two groups included performance of the combined analysis of variance (mixed between-within subjects ANOVA). The results were considered statistically significant when probability of the null hypothesis was lower than 5% ($p < 0.05$).

Results

Basic characteristics of patients are presented in Table 1. Groups were similar according to the gender ($p = 0.598$), diabetes mellitus genetic heritage ($p = 1.000$), active smoking ($p = 0.347$), profession ($p = 0.837$), age ($p = 0.090$), body mass index ($p = 0.773$), disease duration ($p = 0.090$) and laboratory parameters – levels of glycosylated hemoglobin ($p = 0.403$), urea ($p = 0.679$) and creatinine ($p = 0.524$).

At the admission, no statistically significant difference between groups was found either in VAS score ($p = 0.635$) or domains of the EQ-5D-3L score (mobility: $p = 1.000$; self care $p = 1.000$; usual daily activities $p = 0.945$; pain/discomfort $p = 0.962$; anxiety/depression = 1.000; EQ-VAS $p = 0.136$).

The pain intensity, assessed using the VAS scale, before and after every 14-day therapy cycle (PT application, or ALA supplementation) is presented in Table 2. By using combined analysis of variance, a statistically significant influence both of PT and ALA was observed ($\lambda = 0.028$; $p < 0.001$). A statistically significant difference between the effects of those two therapy modalities was observed ($F = 4.78$; $p < 0.05$).

Also, the pain was found significantly reduced in the A group (PT therapy) after the 2nd therapy cycle ($p = 0.032$), as well as before ($p = 0.029$) and after ($p = 0.001$) the 3rd therapy cycle (Table 2).

Quality of life, assessed by EQ-5D-3L questionnaire, is presented in Table 3. There was no statistically significant difference in EQ-5D-3L domains between the groups, neither at the beginning, nor at the end of the study (Table 3). At the end of the study, results in the domains related to mobility, self-care and regular daily activities were not significantly different ($p > 0.05$) compared to the results at the beginning of the study in either group. A statistically significant improvement was found in the domain of pain/discomfort both in the group A ($\eta = 0.54$, $p < 0.001$) and group B ($\eta = 0.57$, $p < 0.001$) and anxiety/depression domain (A group: $\eta = 0.32$, $p = 0.008$; B group: $\eta = 0.22$, $p < 0.019$). EQ-VAS was also significantly improved (groups A and B: $p < 0.05$).

Table 1

Characteristics of patients with diabetic polyneuropathy (DPN)

Characteristics	Group A (n = 30)	GroupB (n = 30)	*p* value
Sex, n (%)			
male	11 (36.67)	13(43.33)	0.598†
female	19 (63.33)	17 (56.67)	
Heredity for DM, n (%)			
yes	13(43.33)	13(43.33)	≈ 1.000†
no	17 (56.67)	17 (56.67)	
Active smoking, n (%)			
yes	8(26.67)	5 (16.67)	0.347†
no	22 (73.33)	25 (83.33)	
Profession, n (%)			
pension	23 (76.76)	22 (73.33)	0.837†
employed	6 (20)	6 (20)	
unemployed	1 (3.33)	2 (6.67)	
Age (years), mean ± SD	63.17 ± 7.68	62.77 ± 8.35	$p = 0.09$‡
Body mass index (kg/m^2), mean ± SD	27.2 ± 4.56	27.2 ± 3.93	0.773*
Duration of diabetes (years), mean ± SD	12.22 ± 7.58	11.70 ± 5.75	$p = 0.09$‡
HbA1c (%), mean ± SD	7.80 ± 1.87	7.30 ± 1.21	0.403*
Urea (mmol/L), mean ± SD	6.51 ± 2.94	6.01 ± 2.08	0.679*
Creatinine (μmol/L), mean ± SD	81.1 ± 22.19	76.33 ± 17.67	0.524*
VAS, mean ± SD	7.67 ± 1.06	7.60 ± 0.89	0.635*
EQ-5D / VAS, mean ± SD	36.57 ± 7.73	39.03 ± 7.24	0.136*

Group A – patients treated by physical therapy; Group B – patients treated by alpha-lipoic acid; VAS – visual analog scale; EQ-5D – European Quality of Life Questionnaire; HbA1c – glycated hemoglobin; DM – diabetes mellitus; SD – standard deviation; * – Mann-Whitney test; † – χ^2; ‡ – *t*-test .

Table 2

Pain estimation in patients with diabetic polyneuropathy by Visual analog scale (VAS)

Parameter	Group A (n = 30) mean ± SD	Group B (n = 30) mean ± SD	*p* value†	*p* value‡
VAS scale I series				
before treatment	7.67 ± 1.06	7.60 ± 0.89	0.635	< 0.05*
after treatment	3.03 ± 1.73	3.73 ± 1.78	0.073	
VAS scale II series				
before treatment	6.43 ± 1.22	6.90 ± 1.00	0.131	< 0.05*
after treatment	2.60 ± 1.50	3.20 ± 1.40	0.032*	
VAS scale III series				
before treatment	5.33 ± 1.32	5.98 ± 1.24	0.029*	< 0.05*
after treatment	1.67 ± 0.84	2.63 ± 0.89	0.001*	

Group A – patients treated by physical therapy; Group B – patients treated by alpha-lipoic acid; * – statistically significant; † – Mann-Whitney test; ‡ – mixed between-within subjects ANOVA test.

Table 3

Proportion of levels 1, 2 and 3 by EQ-5D dimension and type of therapy in patients with diabetic polyneuropathy

EQ-5D DIMENSION		Group A (n = 30), n (%)	Group B (n = 30), n (%)	TOTAL (n = 60), n (%)	*p* value (between groups)
Mobility	Treatment				
L1	before therapy	28 (93.3)	29 (96.7)	57 (95.5)	
	after therapy	28 (93.3)	29 (96.7)	57 (95.5)	Before: $p \approx 1.000$§
L2	before therapy	2 (6.7)	1 (3.3)	3 (5.0)	
	after therapy	2 (6.7)	1 (3.3)	3 (5.0)	After: $p \approx 1.000$§
L 3	before therapy	0 (0.0)	0 (0.0)	0 (0.0)	
	after therapy	0 (0.0)	0 (0.0)	0 (0.0)	
p value (within group) ††		$p \approx 1.000$	$p \approx 1.000$		

Table 3 (*continued*)

EQ-5D DIMENSION		Group A (n = 30), n (%)	Group B (n = 30), n (%)	TOTAL (n = 60), n (%)	*p* value (between groups)
Self- car	Treatment				
L1	before therapy	27 (90)	26 (86.7)	53 (88.3)	
	after therapy	27 (90)	26 (86.7)	53 (88.3)	Before: $p \approx 1.000^{\S}$
L2	before therapy	3 (10)	4 (13.3)	7 (11.7)	
	after therapy	3 (10)	4 (13.3)	7 (11.7)	After: $p \approx 1.000^{\S}$
L 3	before therapy	0 (0.0)	0 (0.0)	0 (0.0)	
	after therapy	0 (0.0)	0 (0.0)	0 (0.0)	
p value (within group) ††		$p \approx 1.000$	$p \approx 1.000$		
Regular daily activities	Treatment				
L1	before therapy	24 (80.0)	23 (76.7)	47 (78.3)	
	after therapy	25 (83.3)	24 (80.0)	49 (81.7)	Before: $0.945^{\S}$
L2	before therapy	5 (16.7)	6 (20.0)	11 (18.3)	
	after therapy	4 (13.3)	5 (16.7)	9 (15.0)	After: $0.936^{\S}$
L 3	before therapy	1 (3.3)	1 (3.3)	2 (3.3)	
	after therapy	1 (3.3)	1 (3.3)	2 (3.3)	
p value (within group) ††		0.317	0.317		
Pain/ Discomfort	Treatment				
L1	before therapy	1 (3.3)	1 (3.3)	2 (3.3)	
	after therapy	10 (33.3)	10 (33.3)	20 (33.3)	Before: $0.962^{\S}$
L2	before therapy	20 (66.7)	19 (63.3)	39 (65.0)	
	after therapy	19 (63.3)	19 (63.3)	38 (63.3)	After: $p \approx 1.000^{\S}$
L 3	before therapy	9 (30.0)	10 (33.3)	19 (31.7)	
	after therapy	1 (3.3)	1 (3.3)	2 (3.3)	
p value (within group) ††		< 0.001*	< 0.001*		
Anxiety/ Depression	Treatment				
L1	before therapy	6 (20.0)	6 (20.0)	12 (20.0)	
	after therapy	14 (46.7)	12 (40.0)	26 (43.3)	Before: $p \approx 1.000^{\S}$
L2	before therapy	14 (46.7)	14 (46.7)	28 (46.7)	
	after therapy	15 (50.0)	17 (56.7)	32 (53.3)	After: $0.870^{\S}$
L 3	before therapy	10 (33.3)	10 (33.3)	20 (33.3)	
	after therapy	1 (3.3)	1 (3.3)	2 (3.3)	
p value (within group) ††		0.008*	0.019*		
EQ-VAS	Treatment				
	before therapy	36.57 ± 7.73	39.03 ± 7.24		$0.136 \pm 0.230^{\dagger}$
	after therapy	75.63 ± 10.11	72.53 ± 9.70		
p value (within group) ††		$p < 0.05$*	$p < 0.05$*		

Group A – patients treated by physical therapy; Group B – patients treated by alpha-lipoic acid;L – level; EQ – MD dimension – European Quality of Life Questionnaire; EQ-VAS – European Quality of Life – Visual Analog Scale; * – statistically significant; † – Mann-Whitney test; § – Fisher's exact test; †† – Willcoxon signed rank test.

Discussion

Research on quality of life and pain reduction in the patients with diabetes mellitus are very popular around the world, which is understandable if one takes into account the participation of diabetes mellitus in the structure of morbidity and mortality and the fact that the timely implementation of appropriate medicamentous and physical therapy achieves not only the prevention of many complications but also significantly improves the quality of life. Studies show that the patients with diabetes and complications have poor quality of life and determinants with the strongest influence are ischemic heart disease, stroke and neuropathy [11].

The usage of the pharmacological preparations in the therapy of DM patients with DSP has been widely studied, while there are not many studies on the effects of physical therapy on quality of life of those patients. The efficiency of the TENS therapy in pain reduction in these patients was confirmed in many studies [12–15] as well as the efficiency of PEMF [16, 17], SG [18, 19] and exercise [20, 21], which is in accordance with our research.

SG exhibits its analgesic effects by inducing hyperemia in the skin and deeper tissues through which it passes. The pain reduction may also be explained by the gate control mechanism, since SG acts on the sensory endings in the skin and suppresses pain in the rear horns of the spinal cord as well as by the release of endogenous opioids [22]. A study by Armstrong et al. [18] showed a statistically significant pain reduction after 4 weeks of SG treatment.

Analgesic effect of TENS therapy is achieved by secretion of endogenous opioids (endorphins, enkephalins) in the central nervous system [23, 24] which inhibit the transmission of pain impulses by closing the door (gate control) for the painful impulses transmitted by C fibers [25]. The TENS therapy showed to be efficient in reducing pain in the patients with DSP in a number of studies. Thus, its use is recommended in treatment of diabetic neuropathy [26–28]. Furthermore, a study by Jin et al. [29] showed that a 4-week TENS therapy induced a statistically significant pain reduction. Our results correlate with previously mentioned study: although the pain was reduced in both groups after 6 months, it was reduced to the greater extent in the patients treated with PT. The difference in pain intensity between the A and B groups in our research was observed after the 2nd therapy cycle and remained until the end of the study.

PEMF has analgesic, neurostimulating, trophic and vasoactive effects [30]. By the mechanisms of depolarization, hyperpolarization and repolarization, it may modulate neuropathic pain and nerve impulses [31]. In contrast to our study, a study by Weintraub et al. [32] showed no statistically significant pain reduction in the DSP patients after the PEMF treatment, but it did show neurodegenerative effect of PEMF. On the other side, a study by Graak et al. [31] did show a statistically significant reduction of pain in the DSP patients after the PEMF administration for 12 weeks, which correlated with our results.

Exercise positively affects pathological factors associated with neuropathy by increasing microvascular vasodilatation, reduction of oxidative stress and increase of neurotrophic factors, but insufficient number of studies explores the effects of exercise on signs and symptoms of DSP. One study showed a statistically significant reduction of pain and improvement of the neurological symptoms in the patients with DSP after 10 weeks of exercise tretment [33], which coincided with the results of our study.

Some studies showed that ALA reduces neuronal sensitivity to pain by selective inhibition of neuronal calcium channels of the T-type [34, 35]. Also, ALA has a clear metabolic effect, improves the microcirculation and has an anti-inflammatory effect. All the above mentioned effects act synergistically in a complex chain of pathophysiological events in the course of the disease [36, 37].

The psychological status of patients with DSP is in correlation with pain, subsequent problems with mobility as well as limitations in an independent functioning [10, 11]. The results of our study showed that the use of PT and the patients' involvement in the rehabilitation program as well as the use of ALA, had a positive effect on improvement of quality of life of diabetics with DSP, which is manifested by the increase in degrees of quality of life measured by EQ-5D.

A study by Bertolloto and Massone [38] showed a statistically significant reduction in pain (measured by VAS scale) in the DM DSP patients after the ALA administration. Pain was also significantly reduced in a study by Patel et al. [39], in which ALA was applied for 6 months.

Investigation of depression and quality of life is an important part of this study because the literature data indicated that the rate of depression in the population of DM patients is 2–3 times higher than usual. Symptoms of depression occur in about 30% of DM patients and major depression in 10% of DM patients [40], which significantly contributed to the development of type 2 diabetes and may accelerate the development of complications caused by the disease [41]. Numerous studies showed that diabetes significantly affects quality of life of patients. The scores on scales of quality of life were becoming lower due to the simultaneous action of underlying disease and other (somatic or psychological) diseases as its resulting complications or as comorbidity.

Many studies indicated lower quality of life and more symptoms of depression in the patients with DM type 2 in comparison with those without DM [42–44]. One study, that explored quality of life by using EQ-5D, showed a significant decline in quality of life of DM patients, with a higher decrease results in a subgroup of patients with DM and complications. The study showed a clear influence of DM type 2 and its complications on reduction of quality of life [45].

By investigating the effect of low frequency magnetic fields on quality of life (measured by the EQ-5D questionnaire) and pain in the DM patients with DSP, Wrobel et al. [46] showed a statistically significant reduction in pain and improved quality of life of patients. Our results correlate with the results of that study, except that in our study, in addition to the magnetic therapy, the electrotherapy (TENS and SG) and exercise were applied. Also, another study [47] demonstrated the effect of exercise on improvement of quality of life.

In this research, we demonstrated the synergistic effect of the applied physical procedures on pain reduction and improvement of quality of life of DM patients with DSP. The application of combined physical procedures showed more pronounced effect in the reduction of pain, but did not give such good results in the improvement of quality of life. This may be a consequence of the application of the generic test for estimating the quality of life or some other factors, such as too short investigation protocol. Because of the numerous comorbidities, the adverse effects and lack of effective pharmacological preparations, application of physical methods in the treatment of these patients should be more prevalent in everyday clinical practice.

Conclusion

Our research showed that both physical therapy and alpha-lipoic acid supplementation have a significant influence on pain reduction and quality of life in the diabetic patients with distal sensorimotor polyneuropathy, but physical therapy has a greater influence on pain reduction.

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The impact of the early tenotomy of Achilles tendon on the length and results of congenital clubfoot severe forms treatment

Uticaj rane tenotomije Ahilove tetive na trajanje i rezultate lečenja teških oblika urođenog krivog stopala

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Abstract

Background/Aim. In this paper we present our modification of the Ponseti method which we have been using for the treatment of severe forms of congenital clubfoot since 2007. The aim of this paper was to determine, on the basis of the analysis of results, the impact of the early tenotomy of the Achilles tendon on the length and results of treatment of severe forms of congenital clubfoot. **Methods.** The study was prospective and lasted from 2007 to 2016 year. It was implemented in the Clinic for Children's Surgery Banjaluka. The Group 1 consisted of the subjects treated by the modified Ponseti method in the period of 9 years (2007–2016). There were a total of 30 subjects (52 feet). There were 20 (67%) of male and 10 (33%) of female subjects. There were 22 (77%) subjects with bilateral and 8 (27%) with unilateral deformity. The Group 2 consisted of the subjects treated by the classic Ponseti method in the period of 9 years (2007–2016). There were a total of 32 subjects (52 feet). There were 26 (81%) of male and 6 (19%) of female subjects. There were 20 (63%) of subjects with bilateral and 12 (37%) with unilateral deformity. We used the Pirani score for: classification of deformity according to the severity, monitoring of results of the correction, determination of indication for tenotomy of the Achilles tendon and for the analysis of results of the treatment. **Results**. The total length of treatment in the Group 1 was from 6 to 9 weeks (mean = 6.71 ± 0.871), and in the Group 2 from 9 to 12 weeks (mean = 9.92 ± 0.882) (r = 0.86; $p = 0.001$). There was no difference in the results of the treatment ($\chi^2 = 2.372$ df = 2 n = 52 $p = 0.936$. **Conclusion.** Applying early tenotomy of Achilles tendon in the treatment of severe forms of congenital club foot significantly shortens the duration of treatment and has no negative impact on the results of treatment.

Key words:
foot deformities; congenital abnormalities; orthopedic procedures; achilles tendon; prognosis.

Apstrakt

Uvod/Cilj. U radu prikazujemo našu modifikaciju Ponseti metode koju koristimo za lečenje teških oblika urođenog krivog stopala od 2007. godine. Modifikacija se sastoji u ranoj tenotomiji Ahilove tetive. Tenotomiju smo radili odmah nakon korekcije metatarzalnog dela stopala. Cilj rada bio je da na osnovu analize rezultata između dve grupe ispitanika utvrdimo uticaj rane tenotomije Ahilove tetive na dužinu i rezultate lečenja teških oblika urođenog krivog stopala. **Metode.** Istraživanje je bilo prospektivno i trajalo je od 2007. do 2016. godine. Sprovedeno je u Klinici za dečiju hirurgiju u Banjaluci. Grupu 1 činili su ispitanici lečeni u periodu 2007–2016. godina modifikovanom Ponseti metodom (n = 30; 52 stopala). Zastupljenost muškog pola bila je 67%, a ženskog pola 33%. Grupu 2 činili su ispitanici lečeni u istom periodu klasičnom Ponseti metodom (n = 32; 52 stopala). Zastupljenost muškog pola u ovoj grupi bila je 81%, a ženskog pola 19%. Piraniev skor koristili smo za: klasifikaciju deformiteta prema težini, praćenje uspeha korekcije, postavljanje indikacija za tenotomiju Ahilove tetive i za analizu rezultata liječenja. **Rezultati.** Ukupno trajanje lečenja u Grupi 1 kretalo se od 6 do 9 nedelja (prosečno 6,71 ± 0,871 nedelja). U Grupi 2 lečenje je trajalo od 9 do 12 nedelja (prosečno 9,92 ± 0,882 nedelja). Trajanje lečenja u Grupi 1 bilo je značajno kraće nego u Grupi 2 (r = 0,86, p = 0,001). Nije bilo razlike između grupa u rezlutatima lečenja ($\chi^2 = 2,372$; df = 2; n = 52; p = 0,936). **Zaključak.** Primena rane tenotomije Ahilove tetive u lečenju teških oblika urođenog krivog stopala značajno skraćuje trajanje lečenja i nema negativan uticaj na rezultate lečenja.

Ključne reči:
stopalo, deformacije; anomalije; ortopedske procedure; ahilova tetiva; prognoza.

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Introduction

Congenital clubfoot is a segmental multifactorial deformity of the lower leg and foot. The deformity is complex and includes tarsal and metatarsal (MT) part of the foot. Basic elements of the deformity are: equines and varus of the tarsal part of the foot, abduction, inversion and cavus of the MT part of the foot.

Three classification protocols accepted and mostly used for the assessment of severity of the deformity are: Dimeglio, Pirani and International Clubfoot Study Group (ICFSG) [1, 2].

The Pirani score (PS) is used for: the clinical assessment of severity of the deformity of children who have not been operated on but are younger than 2 years, for the analysis of results of the treatment of clubfoot treated by the Ponseti method, for monitoring of the correction during the treatment and for the determination of indication for tenotomy of the Achilles tendon (TAT) [3].

The Ponseti method (PM) [2] is used for the treatment of all forms of congenital clubfoot regardless of their severity. The length of treatment is mostly from 5 to 6 weeks, except for the severe forms of deformity where 9-11 weeks are necessary for the correction. The TAT is necessary for the definite correction of the severe forms of deformities. Tenotomy is mostly done in the period from 9 to 11 weeks after treatment. The most common element of the deformity failed to be corrected is equinus of the last part of the foot and the TAT is necessary for its correction [2]. The tenotomy is followed by the immobilization of foot with the upper leg cast for 3 to 4 weeks. The Denis Brown shoes are used to the third year of life in order to prevent the recurrence.

In this paper we show our modification of the Ponseti method (mPM) that we have been using for the treatment of severe forms of the congenital clubfoot since 2007. The application of mPM has the aim to shorten the length of treatment of severe forms of congenital clubfoot. The modification relates to the early tenotomy of the Achillus tendon (eTAT). We performed the tenotomy immediately after the good correction of the MT part of the foot. By application of the classic PM, after the correction of MT port of the foot, the manual correction and immobilization are continued in order to correct the tarsal part of the foot. However, for the purpose of the definite correction, the TAT is necessary, which is done significantly later in comparison to the tenotomy of the subjects treated by the mPM.

There are the various modifications of PM reported in relevant literature [4–19]. Those modifications relate to: the length of period between the correction, the type of material (plastic orthosis, plaster bandages) which are used to maintain the correction and the devices used for the prevention of recurrence. The modification of PM similar to ours has not been described in the available literature. By comparing the results of the group of subjects treated by the classic Ponseti method [3, 20, 21] and subjects treated by the modified Ponseti method, we have established the impact of early tenotomy of Achilles tendon on the length and results of treatment of the severe forms of congenital clubfoot.

We consider that this research will contribute to the treatment of the severe forms of the deformities in terms of achieving the minimum length of treatment and the shortest termination of socioeconomic life of child and parents.

Methods

Subjects

The research was conducted in two types of subjects with the severe form of congenital clubfoot. The Group 1 consisted of the subjects treated by the modified Ponseti method (mPM) in the period of 9 years (2007–2016). There were a total of 30 subjects (52 feet). There were 20 (67%) of male and 10 (33%) of female subjects. There were 22 (77%) subjects with bilateral and 8 (27%) with unilateral deformity. The Group 2 consisted of subjects treated by the classic Ponseti method in the same period of 9 years (2007–2016). There were a total of 32 subjects (52 feet). There were 26 (81%) of male and 6 (19%) of female subjects. There were 20 (63%) of subjects with bilateral and 12 (37%) with unilateral deformity. The study was prospective and lasted from 2007 to 2016 year, implemented in the Clinic for Children's Surgery in Banjaluka. The age of the subjects at the beginning of the research was 15–25 days (mean = 18.25 ± 0.721 days).

Methodology

We used the Pirani score for the classification of deformity according to the severity, monitoring of results of the correction, determination of indication for TAT and the analysis of results of the treatment. For the hindfoot tarsal part score (HS), the following was monitored: posterior crease, equinus and size of the heel, and for the midfoot score (MS): outer border, medial crease and coverage of the head of talus, as shown in Table 1.

The feet with the 3–6 points of Pirani score are classified into the group of severe forms of deformity. By clinical examination of the foot for the analysis of results of the treatment, we determined the Pirani score: at the beginning of the treatment, every 7 days during the treatment, and at the end. The value MS = 0–0.5 indicated the successful correction of the MT part of the foot. In the Group 1 when MS = 0–0.5, regardless of the value of HS, eTAT was performed. In this way we corrected the tarsal part of the foot also. Unlike in the Group 1, in the Group 2 when the values of MS were 0–0.5, we continued with manual correction and immobilization in order to correct the tarsal part of the foot. If the equines was not corrected by the classic Ponseti protocol, the TAT was done when HS ≥ 1. After the tenotomy in both groups, the cast was placed with the feet in positions 20° of extension in the ankle joint and 70° of abduction of the MT part of the foot. The repair of the Achilles tendon was monitored by the ultrasound. In the period following the TAT we did not determine the Pirani score because the manipulation of foot and removal of immobilization could have an impact on the repair of Achilles tendon.

Table 1

Pirani score

Pirani score [PS = HS + MS (0–6 points)]			
Hindfoot score – HS (0-3)	Points	Midfoot score – MS (0–3)	Points
Posterior crease		Outer border	
none	0	flat	0
shallow	0.5	slightly convex	0.5
deep	1	markedly convex	1
Equinus		Medial crease	
absent	0	none	0
correction to 0°	0.5	shallow	0.5
it cannot be corrected	1	prominent	1
Size of the heel		Coverage of the head of talus	
normal	0	good	0
small, empty	1	satisfactory	0.5
		bad	1
HS ≥ 1 indication for TAT (insufficient correction) of equinus and varus)		MS 0–0.5 good correction of metatarsal part of the foot	

TAT – tenotomy of the Achilles tendon.

For the purpose of research we divided the length of treatment (TL) or immobilization into the following periods: the period P1.1 from the beginning of treatment to the correction of MT part of the foot, or to eTAT for the Group 1, the period P1.2 to the correction of MT part of the foot for the Group 2; the period P2 from the beginning of treatment to the tenotomy for the Group 2 and the period P3.1 the duration of immobilization after the tenotomy for the Group 1 and the period P3.2 the duration of immobilization after the tenotomy for the Group 2. The duration of treatment (TL) for the Group 1 was P1.1 + P3.1 and TL for the Group 2 was P2 + P3.2. When PS was 0–0.5 points (HS + MS = 0–0.5) it was considered to be a good correction of the deformity.

Table 2

The values of hindfoot score (HS) in the moment of determination of indication for tenotomy

HS	Number of feet by groups		Total number
	Group 1	Group 2	
0.5	-	9	9
1	11	31	42
1.5	6	10	16
2	13	2	15
2.5	13	-	13
3	9	-	9
Total	52	52	104
Arithmetic mean	2.03	1.03	
Standard deviation	0.70	0.34	

Statistical analysis

The data analysis was done by the software package SPSS 17.0 (SPSS Inc, IBM Corporation, USA). For the descriptive indicators, the arithmetic mean, standard deviation, and coefficient of variation were used. The frequencies were expressed absolutely and relatively (in percentages). The Kolmogorov–Smirnov test was used for the testing of normality of the distribution. The differences between the variables were analysed using the Mann-Whitney test (*U*-test), *t*-test of difference between the proportions for independent samples and *t*-test of difference between the arithmetic means. The connection between the numerical variables was analysed by the Spearman's rank correlation coefficient, and between the nominal variables by the χ^2 test in contingency tables. The statistical significance level was $p < 0.05$.

Results

The results for the length of treatment to and after the tenotomy of the Achilles tendon, to the correction of metatarsal part of the foot and the total length of the treatments

The length of the treatment to the correction of MT part of the foot (P1.1 and P1.2*)* lasted 3–4 weeks for the most of feet (the Groups 1 and 2). In the Group 1, the correction of the MT part of the foot was good to the third week in 30 (57%), to the fourth week in 19 (37%) and to the fifth week in 3 (6%) feet. In the Group 2 ,the correction of the MT part of the foot was good to the third week in 29 (56%) feet, to the fourth week in 18 (37%), to the fifth week in 2 (4%) and in one foot in the sixth, in one foot in the seventh and in one foot in the eight week.

The length of the treatment to TAT (P1-1) in the Group 1 lasted 3–4 weeks. The mean value was 3.50 ± 0.672 weeks. This period corresponds to the period in which the good correction of the MT part of the foot was achieved. The period to TAT (P2) in the Group 2 lasted 6–8 weeks, the mean value 6.65 ± 0.623 weeks. The period to eTAT in the Group 1 was significantly shorter (r = 0.88; $p < 0.001$).

The length of the treatment after TAT (P3.1 and P3.2) in the Groups 1 and 2 lasted 3–4 weeks.The mean value in the Group 1 was mean = 3.21 ± 0.412 weeks, and in the Group 2 3.27 ± 0.448 weeks.

The total length of the treatment in the Group 1 (P1.1 + P3.1) lasted 6–9 weeks, mean = 6.71 ± 0.871 weeks. In the Group 2 the total length of the treatment (P2 + P3.2) lasted 9–12 weeks mean = 9.92 ± 0.882 weeks.

The results and the analysis of the relation between the correction of metatarsal and tarsal part of the foot during the treatment

We controlled the results of the correction based on MS and HS. The correction of MT part of the foot was good when MS = 0–0.5. The value 0–0.5 for HS represents the good correction of the tarsal part of the foot.

In the Group 1 the average value of MS in the third week was 0.81, but in 30 (57%) MS was 0–0.5, and in the fourth week MS was 0.38. After the achievement of the good correction of MT part of the foot we performed TAT. The value of HS at the moment of determination of indication for eTAT (the second, third and the fourth week) was 2.54, 2.33 and 1.8, respectively. The Figure 1 shows the arithmetic means of MS and HS. The averages of MS and HS for the last (the fifth) week were not shown on the chart because they were not representative since they related only to four feet treated in that week.

In the Group 2, the average value of MS in the third week was 0.84 and in 29 (56%) of foot MS was 0–0.5**.** In the fourth week MS = 0.29, in the fifth week MS = 0.11, in the sixth and seventh week MS = 0.02. After achieving a good correction, the immobilization was continued in order to correct the tarsal part of the foot. However, TAT was necessary in order to achieve the definite correction.

The TAT in 21 (40%) of feet was done in the sixth week, while in 28 (54%) of feet in the seventh week and in 3 (6%) of feet in the eight week of the treatment. The average values of HS score in the sixth and seventh week was 1.18 and 1.09, respectively. The averages of MS and HS for the last (sixth) week were not shown on the chart because they are not representative since they relate only to three feet treated in that week (Figure 2).

The results and analysis of hind foot at the moment of the determination of indication for tenotomy

The average HS in the Group 1 at the moment of determination of indication for tenotomy was 2.03, and in the Group 2 was 1.03. The dispersion of scores (HS) in the groups was average and practically the same (variation coefficient was 34%, or 33%). The *t* - test and *U*-test were used to check whether the difference between the arithmetic means between the Groups was accidental, or statistically significant. The first test gave the results $t = 9.30$, meaning that the difference was statistically significant ($p < 0.001$). HS for the tarsal part of foot in the Group 1 was statistically significantly higher than HS in the Group 2. The second test results $U = 334$, $Z = -6.812$ meant that the difference between HS for the tarsal part of foot in the Group 1 in relation to the Group 2 was statistically significant ($p < 0.001$). The average HS values were decreasing in very similar dynamics in the Groups 1 and 2 in the first four weeks of the treatment. After the fourth week the dynamics of decrease was different.

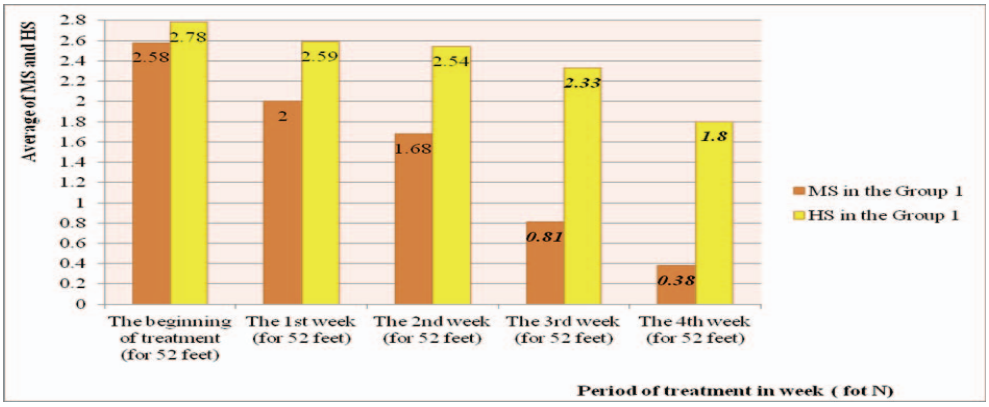

Fig. 1 – Average Pirani scores (MS and HS) of the Group 1.
MS – metatarsal score; HS – hindfoot score.

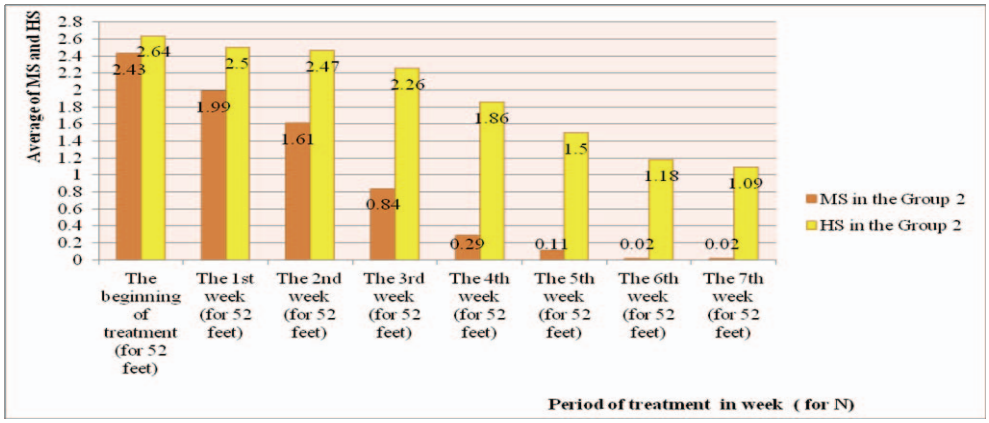

Fig. 2 – Average Pirani scores (MS and HS) of the Group 2.
MS – metatarsal score; HS – hindfoot score.

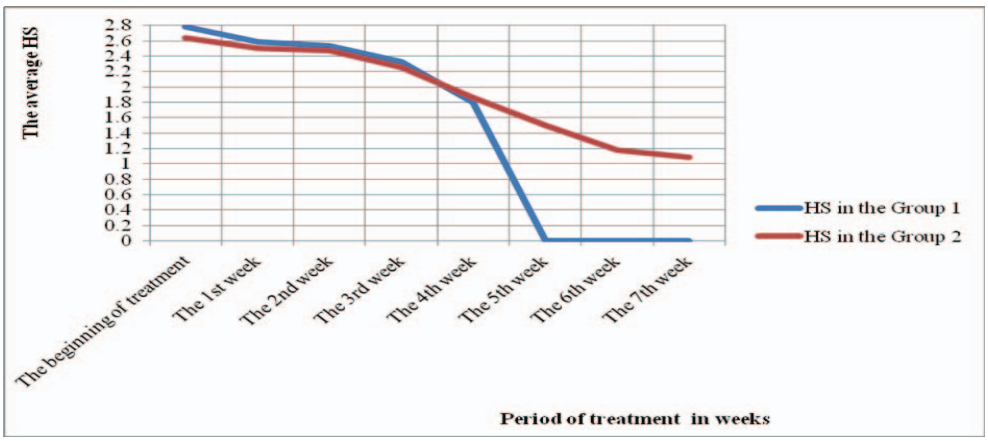

Fig. 3 –The average hindfoot score (HS) during the treatment for the Groups 1 and 2.

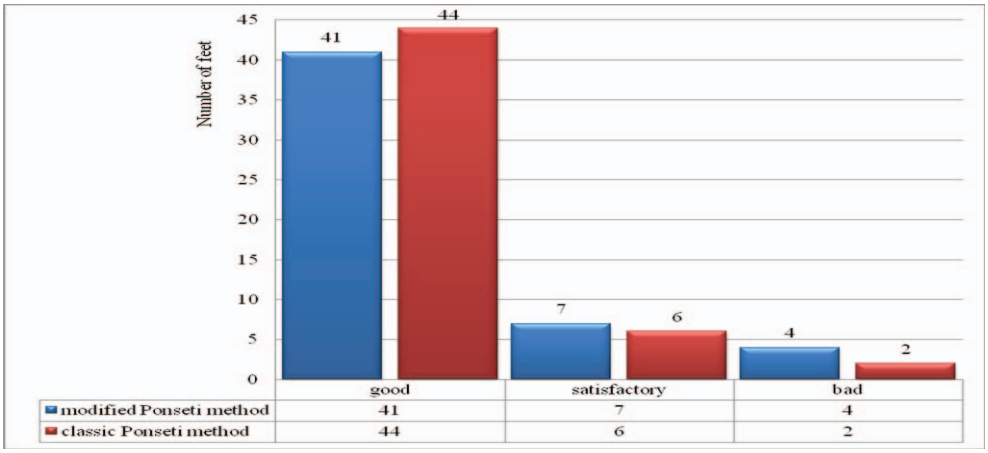

Fig. 4 – Results of treatment by the Groups 1 and 2.

Namely, we performed the eTAT in the Group 1 which was the reason of decrease of HS comparing to the Group 2 in which the tenotomy was done later (Figure 3).

Treatment results

The evaluation of treatment results was done by the Pirani score. The categorization of the results was: good (0–1 points), satisfactory (2–3 points) and bad (3–6 points). There were 41 (78.8%) of good, 7 (13.4%) of satisfactory, and 4 (7.6%) of bad results in the Group 1. There were 44 of good results (84.61%), 6 (11.5%) of satisfactory and 2 (3.8%) of bad results in the Group 2 (Figure 4).

Discussion

The Ponseti method is used for the treatment of congenital clubfoot regardless of the severity of deformity. The treatment of congenital clubfoot with the PM, in most deformites, lasts 5–6 weeks, except for the severe forms the treatment of which lasts 9–11 weeks [15, 16, 2, 3, 20]. In our research the total length of treatment of severe forms of deformities in the Group 2 (PM) was 9.92 weeks which corresponds to the statements of other authors [15, 16, 2, 3, 20]. In the Group 1 (mPM) the treatment lasted 6.71 weeks, which was significantly shorter compared to the Group 2 ($r = 0.86$; $p = 0.001$).

A total of 3–4 weeks were necessary for the correction of metatarsal part of foot in both groups. Other authors [19] publish similar results. In the Group 1, the good correction of the MT part of foot was achieved in 94% of feet to the fourth week. The remaining 6% were 3 feet whose correction was good after five weeks. In the Group 2, the good correction of foot was achieved in 91% of the feet to the fourth week. The good correction of the remaining 9% was achieved during 5–8 weeks. Given that, at the beginning of the treatment we used the same procedure (Ponseti) in both groups. We did not expect the significant difference in time necessary for the good correction of MT part of foot. Between the periods P1.1 and P1.2 (the periods in which the good correction of MT part of foot was achieved), there was no significant difference. After the correction of the MT part of foot, the treatment was different in the Groups 1 and 2.

In the Group 1, we managed to achieve the definite correction of foot by early tenotomy. In 94% of feet, the correction of the MT part of foot was good, and the correction of the tarsal part of foot was unsatisfactory. This is confirmed by the values of HS in the third and fourth week which were 2.53 and 1.8, respectively. After eTAT, the value of HS decreased to 0–0.5 (good correction). We used the MS score,

not the HS one, as the criterion for tenotomy in this group. Namely, when the correction of MT part was good, which we confirmed with MS, we performed eTAT. This was the difference compared to PM when HS was used for the determination of indication for TAT.

In the Group 2, after achieving the good correction of the MT part of foot, TAT was not performed, but it was continued with manual corrections and immobilizations. It was attempted to correct the tarsal part of foot. The values of HS were gradually decreasing from 2.64 at the beggining of the treatment to 1.09. However, we did not manage to achieve a good correction without the tenotomy. Manual correction only mitigated the deformity of tarsal part of foot. In 100% of subjects of the Group 2 the TAT was done in order to definitely correct the tarsal part of foot. Other authors state the same results: Ponseti [3], Laaveg and Ponseti [12], and George and Robin [11]. Tenotomy of our subjects in the Group 2 was done three weeks later compared to the Group 1. Our opinion is that the manual correction of the tarsal part of the foot should not be attempted and that the tenotomy should be immediately performed after the correction of the MT part of foot. According to the statement of the some authors [2, 3, 11, 12, 20] and according to our research, the manual correction of foot in 100% of cases is not successful in the correction of the tarsal part of foot in severe deformities.

By comparing the MS and HS to the fourth week, it can be concluded that the correction of MT part of foot has no significant impact on the correction of the tarsal part of foot (Figure 1 and 2). The correction of the MT part of foot is easier and faster. According to Ponseti [3], the structure of the collagen fibres in the Achilles tendon is different compared to the ligaments of the MT part of foot. The collagen fibres in the Achilles tendon are thicker, stronger and more difficult to stretch. Because of the mentioned, the correction of the tarsal part of foot is more difficult.The average score for metatarsal part of foot (MS) had very similar (parallel) dynamics in the Group 1 compared to the average score in the Group 2. This decrease in both groups was more intensive in the first four weeks of the treatment, i.e., this period was necessary for correction of abduction, inversion and cavus of the MT part of foot. The similar results are stated by: Carroll [4], Abdelgawad et al. [21], and other authors [5–9, 1, 10–12]. The correction of the MT part of foot was the same since the manner of treatment was the same at the beginning.

Between the periods P3.1 and P3.2 there was no significant difference in results of the treatment after the tenotomy between the Groups. The Achilles tendon was repaired in this period, and treatment length could not influence on the treatment outcome. The average value of this period was 3.5 ± 0.182 weeks in our research. The same results are stated by other authors [1, 2, 8–14]. The average age of the subjects in the period of repair of Achilles tendon was $= 2.5 \pm 0.282$ months.

The length of the necessary correction of MT part of foot and periods necessary for the repair of the Achilles tendon cannot be changed, in terms of its shortening, if the treatment is done by PM. Only by early tenotomy the faster correction of the tarsal part of foot is achieved and the length of the treatment is shortened in case of severe forms of the congenital clubfoot.

In the Group 2, there was a greater share of good treatment results, but a smaller share was of the satisfactory and bad results of treatment when compared to the Group 1. This difference is small and accidental. Based on the analysis of the results of treatment there was no difference in the final treatment outcome between the groups.

Conclusion

Applying early tenotomy of Achilles tendon in the treatment of severe forms of congenital bent foot significantly shortens the duration of treatment and has no negative impact on the results of the treatment.

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The glycosylated haemoglobin A1c and albuminuria in patients with type 2 diabetes in the Republic of Srpska: a cross-sectional study

Glikozilirani hemoglobin A1c i albuminurija kod bolesnika sa tip 2 dijabetesom u Republici Srpskoj: studija preseka

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Abstract

Background/Aim. Glycosylated haemoglobin (HbA1c) is currently the gold standard for glucose monitoring in the patients with diabetes. The aim of the present study was to examine the level of success in implementing international guideline targets with regard to glycaemic control in the patients with type 2 diabetes in the Republic of Srpska. This study also aimed to determine the association of albuminuria with the glycaemic control and lipid levels in this patient population. **Methods.** The participating diabetic patients were those registered in the project titled "Estimation of the quality of glycoregulation and presence of vascular complications in the persons with diabetes in the Republic of Srpska." The study was conducted as a cross-sectional study including 1037 patients. HbA1c was determined by a turbidimetric inhibition immunoassay used Roche Diagnostics. Total cholesterol, triglycerides, LDL-C, and HDL-C were determined by reagents from Roche Diagnostics (Roche Diagnostics, Mannheim, Germany) as well as albumin and creatinine in the urine. **Results.** Mean value for HbA1c was 7.35 ± 1.61% (57 ± 18 mmol/moL). The 49.46% of all participants achieved target values of HbA1c (< 7% or 53 mmol/moL) and 40.30% had albumin to creatinine ratio (ACR) < 30 mg/g. When the patients were divided according to HbA1c (with HbA1c < 7%, 519 patients, and HbA1c ≥ 7.0%, 510 patients) the ACR values were different between these groups (39.00 vs. 79.50, $p < 0.001$). We found no significant difference with respect to lipid status between the groups. **Conclusion.** The patients with type 2 diabetes in the Republic of Srpska, in a large percentage, did not meet targets for glycaemic control. Improvements are necessary in the treatment and maintenance of this disease process to ensure achievement of goals in management of diabetes, which in turn would decrease longstanding complications of type 2 diabetes.

Key words:
diabetes mellitus, type 2; glycated hemoglobin a; proteinuria; lipids.

Apstrakt

Uvod/Cilj. Glikozilirani hemoglobin (HbA1c) je trenutno zlatni standard za praćenje glikoregulacije kod bolesnika sa dijabetesom. Cilj našeg istraživanja bio je da se ispita da li je postignut željeni cilj u pogledu kontrole glikemije kod bolesnika sa dijabetesom tip 2 u Republici Srpskoj u skladu sa međunarodnim smernicama i da li neregulisana glikemija utiče na pojavu perzistentne albuminurije i poremećaj lipidnog statusa kod osoba sa tip 2 dijabetesom. **Metode.** Analizirani uzorak su činili bolesnici sa tip 2 dijabetesom koji su bili uključeni u projekat pod nazivom "Procena kvaliteta glikoregulacije i prisustva vaskularnih komplikacija kod osoba sa šećernom bolešću u Republici Srpskoj". Istraživanje je sprovedeno kao studija preseka u 2013/2014, sa učešćem 1037 bolesnika. Za merenje HbA1c je korišten imunoinhibicijski test, Roche Diagnostics. Ukupni holesterol, trigliceridi, LDL-C i HDL-C izmereni su reagensima Roche Diagnostics (Roche Diagnostics, Mannheim, Nemačka) kao i albumin i kreatinina u urinu. **Rezultati.** Srednja vrednost za HbA1c bila je 7,35 ± 1,61% (57 ± 18 mmol/moL). Od svih ispitanika samo 49,46% postiglo je ciljne vrednosti HbA1c (< 7% ili 53 mmol/moL), a 40,30% imalo je normoalbuminuriju, odnosno, odnos albumina i kreatinina (ACR) u urinu < 30 mg/g. Kada su bolesnici podeljeni prema HbA1c (HbA1c < 7%, broj bolesnika 519 i HbA1c > 7.0%, broj bolesnika 510) dobijena je značajna razlika u ACR vrednostima (39,00 *vs* 79,50; $p < 0,001$), ali nije pronađena značajna razlika između ove dve grupe bolesnika u odnosu na lipidni sta-

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tus. **Zaključak.** Kod osoba sa tip 2 dijabetesom, u Republici Srpskoj, u velikom procentu nisu postignute ciljne vrednosti glikemije. Naši rezultati ukazuju na neophodne dodatne mere kojima bi se postigli ciljevi međunarodnih i lokalnih smernica, a kojima bi se smanjile učestale komplikacije tip 2 dijabetesa.

Ključne reči:
dijabetes melitus, insulin-nezavisni; hemoglobin a, glukozilovan; proteinurija; lipidi.

Introduction

Diabetes Mellitus is the most common metabolic disorder and one of the biggest health problems of the 21st century. The estimated worldwide prevalence of diabetes mellitus was 415 million in 2015 and it is expected to rise to 642 million by 2040 [1]. Diabetes requires continuous medical care with multifactorial strategies aimed at preventing and decreasing risks of chronic complications [2]. Chronic hyperglycaemia is tied to long lasting consequences that manifest in various organ dysfunctions, especially kidneys and blood vessels [1]. Diabetes is the most common cause of kidney failure in the world [3–5], and it is estimated that diabetes increases the risk of end-stage renal disease (ESRD) approximately 12-fold [6].

Additionally, diabetes was found to be associated with a 2–4 fold increased risk of myocardial infarction, congestive heart failure, and peripheral arterial disease [7–9]. Many studies have shown a strong link between nephropathy and atherosclerotic cardiovascular disease in patients with type 1 and type 2 diabetes [10, 11].

Glycaemic control is fundamental to the management of diabetes. Several studies have shown the benefit of intensive glycaemic control in reducing the frequency of diabetic microvascular complications, such as retinopathy and nephropathy. The results of long term follow-up of patients with diabetes, who were enrolled in the earlier trials, showed that initial strict glycaemic control led to a reduction in incidence of microalbuminuria and cardiovascular disease outcomes when compared to standard therapies [12–14]. Glycosylated haemoglobin (HbA1c) is currently the gold standard for glucose monitoring in the patients with diabetes and was increasingly adopted as a criterion for diabetes diagnosis.

HbA1c below or around 7% were shown to reduce microvascular and neuropathic complications of type 1 and type 2 diabetes. The American Diabetes Association (ADA) recommends a HbA1c level of less than 7.0% as the standard glycaemic treatment goal [15].

The aim of the present study was to examine the level of success in implementing international guideline targets with regard to glycaemic control in the patients with type 2 diabetes in the Republic of Srpska. This study also aimed to determine the association of albuminuria with glycaemic control and lipid levels in this patient population.

Methods

Subjects

The participating diabetic patients were those registered in the project titled ''Estimation of quality of glycoregulation and presence of vascular complications in the persons with diabetes in the Republic of Srpska." The study was conducted as a cross-sectional study in 2013/2014, including 1,037 patients, age 18 and above, who were randomly selected from the registers for diabetes mellitus type 2 in 13 Health Centres in the Republic of Srpska.

Criteria for participation in the study included that the patients needed to be registered in the population register for diabetes in The Republica of Srpska and in available Health Center registers for family medicine, be at least 18 years old, and had lived in the Republic of Srpska over the year preceding the study. This study excluded pregnant women diagnosed with gestational diabetes and patient whose psychophysical status presented a difficulty in communication.

For the measurement of biochemical parameters, the blood and urine samples were taken in the morning between 7:00 and 10:00 a.m. after 12–14 h of fasting. The patients were diagnosed with albuminuria with an albumin-to-creatinine ratio equal to or greater than 30 mg/g creatinine.

The study was approved by the National Ethics Committee. Written consent was obtained from the participating subjects.

Biochemical analyses

HbA1c, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined by reagents from Roche Diagnostics (Roche Diagnostics, Mannheim, Germany) as well as albumin and creatinine in the urine, according to the manufacturer's instructions. Briefly, for HbA1c, anticoagulated whole blood is haemolyzed prior to determination of HbA1c by a turbidimetric inhibition immunoassay, liberated hemoglobin (Hb) in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum, and measured biochromatically. The instrument calculates the % of HbA1c from the HbA1c/Hb ratio according to a user-selected protocol [16]. The reference values for HbA1c were < 6.5% or 48 mmol/moL, TC < 5.0 mmol/L, HDL-C ≥ 1.16 ♂/1.30♀ mmol/L, LDL-C < 2.6 mmol/L, TG < 1.7 mmol/L and albumin-to-creatinine ratio < 30 mg/g. The urine albumin to creatinine ratio (ACR) was used as the index of urinary albumin excretion. Conventionally, subjects with ACR < 30 mg/g were defined as having normal albuminuria, microalbuminuria was defined as ACR ≥ 30 mg/g and macroalbuminuria as ACR ≥ 300 mg/g [17].

Numerous aspects must be considered when setting glycaemic targets. The ADA proposes optimal targets, but each target must be individualized to the needs of each patient and his or her disease factors. The recommendations include blood glucose levels that appear to correlate with achievement of an HbA1c of 7% (53 mmol/moL). The current guideline recommendations for the Republic of Srpska patients with diabetes is HbA1c < 7.0% (53 mmol/moL) to

prevent complications. Reliability of the measurement results was regularly checked through assessment of appropriate controls and application of internal and external quality control principles. HbA1c data were collected and analyzed for the study using percentage values and converted to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) values in mmol/mol, which are also cited for the key values reported in the text and tables.

Statistics

All calculations were performed using the SPSS V. 22.0 (IBM SPSS Statistics, Version 22.0. Armonk, NY: IBM Corp.). The Kolmogorov-Smirnov test was used to test normality. The outliers were identified and omitted using the Tukey's method. The values are presented as median and interquartile range (Q1/Q3) and mean ± standard deviation. The Mann-Whitney *U*-test was used to compare data between the groups. We analyzed different groups according to albuminuria by the ANOVA test. The relations between the variables were determined using the Spearman's rank-order correlation test.

Results

The study sample consisted of 1037 subjects, 44.5% men and 55.5% women, equally selected from rural and urban areas. Average age was 64, the majority was ≥ 65 years old, 47.9% of subjects. The highest percentage were the patients with a diabetes diagnosis of less than 5 years (42.5%). The characteristics of patients with type 2 diabetes were shown in Table 1. Mean value for HbA1c was 7.35 ± 1.61% (57 ± 18 mmol/moL) [the lowest 4% (20 mmol/moL), the highest 14.50% (135 mmol/moL)]. Of all participants, 49.46% achieved target values of HbA1c, < 7% or 53 mmol/moL. The distribution of HbA1c values was shown in Figure 1. Elevated (≥ 5 mmol/L) was evident in 75.60% of subjects, whereas a low level of HDL-C (< 1.15♂/1.29♀ mmol/L) was present in 55.9% of subjects and high LDL-C (≥ 2.60 mmol/L) was recorded in 86.0% of subjects. The high values of TG (≥ 1.70) were recorded in 54.3% of subjects.

The subjects were divided into two groups, those with HbA1c < 7% (53 mmol/moL), 519 subjects, and those with HbA1c ≥ 7.0% (53mmol/moL), 510 subjects and we found that there was a statistically significant difference with respect to albuminuria between the two groups, whereas we found no statistically significant difference with respect to the lipid status between the groups. The values of biochemical parameters in different HbA1c groups are shown in Table 2.

Table 1
Clinical characteristics of patients with type 2 diabetes mellitus

Parameter	Patients (n = 1,037)
Age (years)	64 (58/71)
Gender (female)	55.5%
Therapy	100%
BMI (kg/m^2)	30.00 (27.00/30.00)
Waist circumference, female (cm)	101.50 (94.00/110.00)
Waist circumference, male (cm)	104.00 (98.00/111.00)
Systolic pressure (mmHg)	140 (130/160)
Diastolic pressure (mmHg)	85 (80/90)
HbA1c < 7.0% (53 mmol/moL)	49.46%
Albumin to creatinine < 30 mg/g	40.30%
HbA1c, % (mmol/L)	7.35 ± 1.61 (57 ± 18)

The values are presented as medians and interquartile range (Q1/Q3) and mean ± standard deviation; n – number of patients; ACR – albumin to creatinine ratio; HbA1c – hemoglobin A1c.

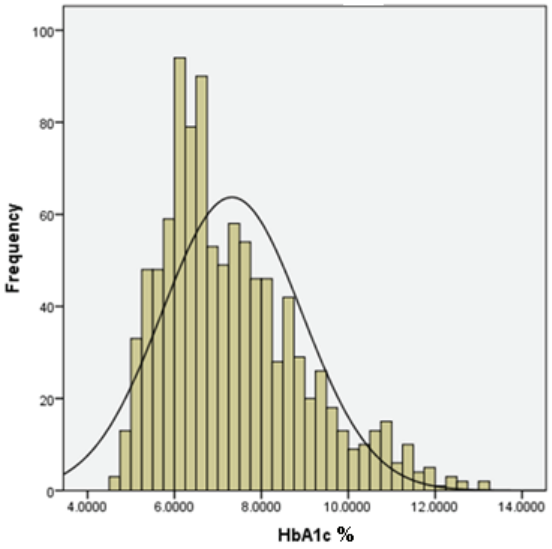

Fig. 1 – Distribution of hemoglobin A1c (HbA1c) values.

Table 2
Parameters in different HbA1c groups in the patients with type 2 diabetes mellitus

Parameter	HbA1c < 7.0% (53 mmol/moL)		HbA1c ≥ 7.0% (53 mmol/moL)		*p*
	n	median (Q_1/Q_3)	n	median (Q_1/Q_3)	
HbA1c (%)	519	6.13 (5.67/6.50)	509	8.25 (7.56/9.33)	< 0.001
or mmol/moL		43 (38/48)		67 (59/78)	
ACR (mg/g)	519	39.00 (10.00/124.75)	510	79.50 (18.00/199.25)	< 0.001
TC (mmol/L)	517	5.76 (4.89/6.66)	516	5.72 (4.93/6.63)	0.738
HDL-C (mmol/L)	520	1.28 (1.07/1.53)	513	1.27 (1.07/1.51)	0.751
LDL-C (mmol/L)	512	3.85 (3.07/4.59)	510	3.72 (3.05/4.51)	0.523
TG (mmol/L)	515	1.82 (1.29/2.52)	512	1.78 (1.31/2.57)	0.640

The values are presented as medians and interquartile range (Q1/Q3). The Mann-Whitney *U*-test was used to compare data between groups. ACR – albumin to creatinine ratio; HbA1c – hemoglobin A1c; TC – total cholesterol; HDL-C – high density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; TG – triglycerides.

Table 3

Biochemical parameters in different albuminuria groups

Parameters	Groups	n	Median (Q_1/Q_3)	Difference between groups	*p*
ACR (mg/g)	1 (ACR < 30 mg/g)	402	9.00 (6.00/17.00)	1 : 2/1 : 3	< 0.05
	2 (ACR 30–299 mg/g)	508	106.50 (59.00/171.75)	1 : 3	< 0.05
	3 (ACR ≥ 300 mg/g)	115	497.00 (361.00/ 885.00)	1:2	
HbA1c (%) or mmol/moL	1 (ACT < 30 mg/g)	405	6.60 (5.93/7.78) 53 (41/62)	2 : 3	
	2 (ACR 30–299 mg/g)	502	7.20 (6.30/8.50) 55 (45/69)	2 : 1	
	3 (ACR ≥ 300 mg/g)	121	7.43 (6.45/ 8.73) 58 (47/72)	3 : 1	
TC (mmol/L)	1 (ACR < 30 mg/g)	404	5.76 (5.00/6.65)	-	
	2 (ACR 30–299mg/g)	506	5.68 (4.85/6.60)	-	
	3 (ACR ≥ 300 mg/g)	123	5.95 (4.93/ 6.80)	-	
HDL-C (mmol/L)	1 (ACR < 30 mg/g)	404	1.29 (1.08/1.53)	-	
	2 (ACR 30–299 mg/g)	506	1.27 (1.07/1.53)	-	
	3 (ACR ≥ 300 mg/g)	123	1.28 (1.06/ 1.50)	-	
LDL-C (mmol/L)	1 (ACR < 30 mg/g)	400	3.91 (3.18/4.67)	1 : 2	
	2 (ACR 30–299 mg/g)	501	3.68 (2.98/4.44)	2 : 1	
	3 (ACR ≥ 300 mg/g)	121	3.78 (2.98/ 4.57)	-	
TG (mmol/L)	1 (ACR < 30 mg/g)	405	1.87 (1.34/2.52)	-	
	2 (ACR 30–299 mg/g)	502	1.75 (1.24/2.46)	2 : 3	
	3 (ACR ≥ 300 mg/g)	120	1.82 (1.42/2.96)	3 : 2	

The values are presented as medians and interquartile range (Q1/Q3). The ANOVA test was used. ACR – albumin to creatinine ratio; HbA1c – Hemoglobin A1c; TC – total cholesterol; HDL-C – high density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; TG – triglycerides; n – number of subjects.

We subdivided the subjects into three groups with respect to urine albumin concentrations. The group 1 (n = 402) had an ACR < 30 mg/g, the group 2 (n = 508) with albuminuria and ACR > 30–299 mg/g, and the group 3 (n = 115) with ACR ≥ 300 mg/g. We analyzed these groups to determine if differences exist among groups with respect to their lipid statuses and HbA1c values. Biochemical parameters in different albuminuria groups are shown in Table 3.

In our group of patients, there was no correlation between the HbA1c and the ACR index nor with the lipid parameters (results not shown).

Discussion

In this study, we evaluated glycaemic control, albuminuria and the lipid levels in the patients with type 2 diabetes. These are all significant risk factors for complications in these patients. Our data showed that the mean values HbA1c in the patients with type 2 diabetes in the Republic of Srpska (7.35%) were very similar to the values in studies conducted in several other European countries [18, 19]. Also, the prevalence of patients who achieved the target values of HbA1c (< 7%) approaches the reported prevalence in other European countries, i.e., 49.15% vs. 53.6% [19] and 49.15% vs. 55.7% [20]. However, the numbers are still unsatisfactory in striving to achieve the full goals in the prevention of complications in the patients with diabetes. Regardless of advances in the treatment of type 2 diabetes, the studies showed that many patients remain in poor glycemic control [20], which could be attributed to the complex pathophysiology of diabetes as well as noncompliant behaviour of patients with respect to life style changes (nutrition and physical activity) as well as continuation of prescribed therapies. Strategies in following glycaemia and its effects are very important, given that type 2 diabetes is the leading cause of cardiovascular disorders.

The results of our study showed that there was a significant difference with respect to ACR between the groups of patients with the target HbA1c (< 7.0%) and the groups above the target HbA1c values (≥ 7.0 %) ($p < 0.001$). This suggests that the successful therapy, i.e., achieving the target value of HbA1c (< 7.0%), was also successful in reducing microalbuminuria, which is certainly associated with a long-term risk reduction for microvascular complications, including the kidney failure. The results do confirm the knowledge gained from the studies that the patients with type 2 diabetes with higher HbA1c have the higher values of ACR [21].

However, between our two groups, there was no statistically significant difference with respect to the lipid status. We expected a difference between the lipid parameters, given that there was a high presence of pathologic parameters in the lipid statuses of our subjects as well the results of other studies that showed that persistent high levels of albuminuria were correlated with the elevated levels of total serum cholesterol as well as LDL-C, i.e., that albuminuria preceded the development of hypercholesterolemia [22]. We were especially interested in the analysis of the relationship between HDL-C and albuminuria, since many studies showed that these two parameters were implicated in the development of chronic kidney disease [23]. It is interesting that our study did not confirm the results reported in the study by Kim et al. [24] and Sun

et al. [25], where the patients with diabetes and albuminuria had the lower HDL-C values compared to the ones with normal albuminuria. One of the reasons could be that patients were not separated by gender when the analysis of correlation between albuminuria and lipid parameters was done, i.e., with LDL-C and HDL-C that were significantly better in the women. Some studies showed gender differences in the relationship between albuminuria and dyslipidemia [26, 27].

The only statistically significant difference we found was in the values of triglycerides after we subdivided our patients into the groups based on intensity of albuminuria and those results confirmed the results of numerous studies that found that the high TG was associated with ACR in the patients with diabetes [28–30].

This study has limitations. First, the specific types of antihypertensive medication that may influence albuminuria or renal function, such as angiotensin converting enzyme inhibitors or angiotensin II receptor blockers were not taken into account. Second, a number of patients were taking statins, which could have given a skewed picture of the correlation between microalbuminuria and cholesterol (TC, LDL-C and HDL-C). Third, we did not conduct triple measurements of parameters, in different periods of time, due to financial and technical reasons, which could have an influence on data collected.

Conclusion

The patients with type 2 diabetes in the Republic of Srpska, in a large percentage, did not meet targets for glycaemic control. The patients achieving HbA1c < 7% could have reduction of cardiovascular disease risk by reducing their ACR index. Our results do show that there is a large subset of patients with risk factors for complications associated with diabetes, such as the risk of developing renal dysfunction and cardiovascular disease. Improvements are necessary in the treatment and maintenance of this disease process to ensure the achievement of goals in management of diabetes, which in turn would decrease longstanding complications of type 2 diabetes, mortality and costs of treatment.

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Clinical performances of EuroSCORE II risk stratification model in the Serbian cardiac surgical population: a single centre validation study including 10,048 patients

Kliničke performanse modela za stratifikaciju operativnog rizika EuroSCORE II kod kardiohirurških bolesnika u Srbiji: studija provere na 10 048 bolesnika operisanih u jednom centru

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Abstract

Background/Aim. The EuroSCORE II has recently been developed with an idea to provide better accuracy in prediction of perioperative mortality in the patients who underwent open heart surgery. The aim of this study was to validate clinical performances of the European System for Cardiac Operative Risk Evaluation (EuroSCORE) II risk stratification model in the Serbian adult cardiac surgical population undergoing open heart surgery. **Methods.** The EuroSCORE II values on 10,048 consecutive patients undergoing major adult cardiac surgery from 1st January 2012 to 31st March 2017, were prospectively calculated and entered the institutional database. The discriminative power of the model was tested by calculating the area under the receiver operating characteristic curve (AUC). The calibration of the model was assessed by the Hosmer-Lemeshow (H-L) statistics and the observed to expected (O/E) mortality ratio. The patients with the EuroSCORE II values of 0.5–2.50%, > 2.50–6.50%), and > 6.50% were defined to be at low, moderate, and high perioperative risk, respectively. **Results.** The observed in-hospital mortality was 3.86% (388 of 10,048) and the mean predicted mortality by the EuroSCORE II was 3.61%. The discriminatory power was very good for the entire cohort as well as for all subgroups [coronary, valve(s), combined (coronary plus valve), aortic and other] of performed cardiac procedures (all AUCs > 0.75). The H-L test confirmed good calibration only for category other cardiac procedures. The O/E mortality ratio confirmed good calibration for the whole sample [O/E ratio 1.07, 95% confidence interval (CI) 0.96–1.18] and for all subgroups of performed cardiac procedures, excluding significant underprediction of mortality for aortic surgery (O/E ratio 1.64; 95% CI 1.31–1.97). The EuroSCORE II overestimated perioperative risk in a low and underestimated perioperative risk in a high risk group, with acceptable discrimination (both AUCs = 0.72). On the contrary, the O/E mortality ratio confirmed good calibration for all three subcategories of high risk group. **Conclusion.** The results of our study confirmed acceptable overall performances of the EuroSCORE II risk stratification model in terms of discrimination and the accuracy of model when applied to the contemporary Serbian cardiac surgical cohort undergoing open heart surgery at our Institute.

Key words:
mortality; predictive value of tests; risk assessment; thoracic surgical procedures.

Apstrakt

Uvod/Cilj. EuroSCORE II je razvijen nedavno sa idejom da se obezbedi bolja tačnost u predviđanju perioperativnog mortaliteta bolesnika podvrgnutih operacijama na otvorenom srcu. Cilj rada je bio da se provere kliničke performanse modela za stratifikaciju operativnog rizika u kardiohirurgiji – EuroSCORE II (Evropski sistem za procenu kadiohirurškog operativnog rizika) kod odraslih bolesnika u Srbiji kod kojih se izvode kardiohirurške procedure. **Metode.** Vrednosti EuroSCORE II za 10 048 uzastopno operisanih (od 1. januara 2012. do 31. marta 2017. godine) odraslih kardiohirurških bolesnika prospektivno su izračunate i unete u bazu podataka Instituta za kardiovaskularne bolesti

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"Dedinje" u Beogradu. Diskriminaciona snaga modela testirana je izračunavanjem površine ispod *receiver operating characteristic* (ROC) krive (AUC). Kalibracija modela je bila procenjena upotrebom Hosmer-Lemeshow (H-L) testa i odnosom između zabeleženog i očekivanog (O/E) mortaliteta. Bolesnici sa vrednostima EuroSCORE II od 0,5% do 2,5% definisani su kao bolesnici sa niskim operativnim rizikom, sa skorom preko 2.5% do 6.5 % sa umerenim, a preko 6,5% sa visokim operativnim rizikom. **Rezultati.** Zabeleženi bolnički mortalitet bio je 3,86% (388 od 10,048), a srednja vrednost mortaliteta predviđenog EuroSCORE-om II iznosila je 3.61%. Diskriminatorna snaga modela je bila vrlo dobra za ceo uzorak, kao i za sve podgrupe [koronarna, valvularna, kombinovana (koronarna plus valvularna) hirurgija, hirurgija grudne aorte i ostalo] izvedenih kardiohirurških procedura (sve AUCs > 0.75). H-L testom potvrđena je dobru kalibracija samo za kategoriju 'druge procedure'. Primenom O/E odnosa potvrđena je dobra kalibracija za ceo uzorak [O/E odnos 1.07, 95% interval pouzdanosti (CI) 0.96–1.18], kao i za sve podgrupe izvedenih kardiohirurških procedura, osim značajnog potcenjivanja mortaliteta u hirurgiji grudne aorte (O/E odnos 1.64; 95% CI 1.31–1.97). EuroSCORE II precenio je operativni rizik u grupi niskog rizika, i potcenio operativni rizik u grupi viskog rizika (O/E odnos mortaliteta), sa prihvatljivom diskriminacijom za obe grupe (AUC = 0.72). Naprotiv, O/E odnos mortaliteta potvrdio je dobru kalibraciju za sve tri potkategorije grupe viskog operativnog rizika. **Zaključak.** Rezultati naše studije potvrđuju prihvatljive opšte performanse (diskriminaciju i kalibraciju) EuroSCORE II modela za stratifikaciju operativnog rizika u kardiohirurgiji, primenjenog na uzorak kardiohirurških bolesnika u Srbiji operisanih u našem Institutu, nakon uvođenja modela u upotrebu.

Ključne reči:
mortalitet; testovi, prognostička vrednost; rizik, procena; hirurgija, torakalna, procedure.

Introduction

Despite the progress in preoperative screening, myocardial protection, surgical techniques, and intensive care unit treatment, the open-heart procedures still carry a risk of mortality and significant morbidity. Risk adjusted perioperative mortality rate following cardiac surgery has been widely used as an indicator of quality of care as well as for comparison of outcomes among institutions and surgeons. In order to assess patients' perioperative risk, several scoring systems were developed over past two decades. Of these risk scores, the Society for Thoracic Surgeons (STS) Predicted Risk of Mortality (PROM) score and the European System for Cardiac Operative Risk Evaluation II (EuroSCORE II) are the most widely used worldwide and they were included in clinical guidelines [1]. The EuroSCORE II [2] has recently been developed as an updated version of the old logistic EuroSCORE with an idea to provide better accuracy (calibration) in prediction of perioperative mortality which aged model overestimated by two- to threefold [2, 3]. Four cardiac centers from Serbia [2] contributed to the EuroSCORE II database (22,381 patients), with more than 1,000 patients [Institute for Cardiovascular Diseases – (ICD) Vojvodina – more than 300 patients [4], ICD Dedinje – almost 500 patients, data for other two centers were approximately calculated]. The initial results of EuroSCORE II validation in the Serbian cardiac cohort were reported by two centers [(ICD Vojvodina [5], and ICD Dedinje [6]), including 1,247 and 1,864 patients (who were operated during 2012), respectively]. The results of coronary artery bypass grafting (CABG), valve(s) and combined [CABG plus valve(s)] surgery were studied in both manuscripts. Although a cohort size of both databases was relatively small, the authors were not able to recruit samples large enough to perform a subanalysis of more specific procedures [i.e. aortic valve replacement (AVR), mitral valve replacement (MVR) or mitral valve repair (MVr) surgery, those procedures combined with CABG, etc]. Therefore, the aim of our study was to validate the EuroSCORE II performances in the contemporary cardiac surgical cohort, large enough to allow more comprehensive analysis of all types of cardiac procedures which were performed during the period of five years.

Methods

The EuroSCORE II values were prospectively calculated using the web-based system (http://www.euroscore.org – this site also include definitions of all EuroSCORE II variables), and .stored in the institutional database for a series of 10,048 consecutive patients who underwent adult (≥ 18 years of age) cardiac surgery at the Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia, from 1st January 2012 to 31st March 2017. The patients with a postinfarction ventricular septal rupture were excluded from the study due to a low number of patients with this complication included in the developmental database of EuroSCORE II [2, 7, 8]. Only the first procedure for each patient was entered the registry while any other operation performed during the same in-hospital stay was coded as a complication. The primary end-point for the study was in-hospital mortality (any-cause postoperative death occuring before discharge from the index hospitalization). The patients with the EuroSCORE II values of 0.5%–2.50% > 2.50%–6.50%; and > 6.50% were defined to be at low, moderate, and high perioperative risk, respectively. The high-risk patients were further divided into three categories – higher, very high and extremely high perioperative risk with the EuroSCORE II values of > 6.50%–13.50% > 13.50%–20.00% and > 20.00%, respectively. The Institutional Ethics Committee approved the study and a requirement for informed written consent was waived due to the fact that patients' identities were masked.

The statistical analyses were performed by using the statistical package SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). The categorical variables were expressed as percentages and continuous variables were expressed as mean ± standard deviation (SD). The statistical analyses were performed by the Fisher's exact test, or the χ^2 test for the cate-

gorical variables and by the *t*-test for the continuous variables. A *p*-value lower than 0.05 was considered significant.

The performance of the EuroSCORE II (for the whole cohort as well as for all subgroups) was analysed focusing on the discrimination power and calibration. Discrimination measures the capacity of the model to distinguish between the patients who will develop an event (in this case perioperative death) and those who will not, namely, to differentiate the low-risk from the high-risk patients. Discrimination can be assessed by the area under the receiver operating characteristic curve (AUC). The AUC is a percentage of randomly drawn pairs (meaning one death and one survivor patient-pairs) for which it is true that a patient who died had a higher risk score than a patient who survived. The discriminative power is thought to be excellent if the AUC is > 0.80, very good if > 0.75 and good (acceptable) if > 0.70 [9].

Calibration refers to the agreement between observed events and predicted probability of occurrence of these events. The Hosmer-Lemeshow (H-L) goodness-of-fit test is the most popular test to validate calibration, measuring the differences between the observed and expected outcomes over deciles of risk. A well-calibrated model gives a corresponding *p*-value > 0.05 [10]. We also evaluated the EuroSCORE II calibration using the observed to expected (O/E) mortality ratio. Ideally, this ratio equals one (the observed mortality equals expected mortality, thus the predictive model is perfectly calibrated). A value above one means that the model underestimates mortality, a value below one means that the model overestimates mortality. If the 95% confidence interval (CI) of the O/E mortality ratio includes the value of 1.0, the model is well calibrated [10].

Results

A total of 10,048 patients were identified to fulfill the study criteria [patients < 18 years of age and patients with postinfarction ventricular septal defect (VSD) were excluded]. The operative details and the patients characteristics (EuroSCORE II variables) of our study population and those of the EuroSCORE II are presented and compared in Table 1. There were no single missing data reffering to the variables necessary for the EuroSCORE models risk calculation. The following subgroups procedures were performed: CABG surgery – 5,228 (52.03%); valve(s) surgery (surgery of one or more valves) – 2,305 (22.94%); combined cases (CABG and valve(s) surgery) – 1,569 (15.62%); aortic (thoracic aorta) surgery – 747 (7.43%) and other major cardiac procedures – 199 (1.98%).

The discriminatory and calibration abilities of EuroSCORE II pulled out from the initial studies of the Serbian cardiovascular centers [5, 6] are summarised in Table 2, as well as the ICD "Dedinje" data including over 10,000 patients. The in-hospital mortality observed in our 5-year sample was 3.86% (388 out of 10,048), while the EuroSCORE II predicted mortality of 3.61%. For that period, the EuroSCORE II showed very good discriminative power in all categories (all AUCs > 0.75); for the whole cohort and for all subgroups procedures which were performed – CABG, valve(s), combined, aortic, others) (Table 2). The EuroSCORE II discriminative power was also good for almost all (12 out of 14) more specific procedures [aortic valve surgery, mitral valve surgery, multiple valve surgery as well as for those procedures combined with CABG surgery, excluding MVR plus tricuspid valve surgery (TVs) and MVr plus TVs plus CABG) (Table 3). Although the H-L statistics failed to confirm the overall and subgroups good calibration (except in the category 'others': the H-L *p* value of 0.61), it confirmed a good calibration of EuroSCORE II model for all more specific procedures (Table 3). On the contrary, the O/E mortality ratio confirmed good calibration for the whole sample and for all subgroups of performed cardiac procedures, excluding aortic surgery (a significant underestimation of mortality; O/E mortality ratio = 1.64; 95% CI: 1.31–1.97) (Table 2). The O/E mortality ratio confirmed a good calibration for all more specific procedures (aortic valve surgery, mitral valve surgery, multiple valve surgery, and those procedures combined with CABG surgery), except for MVR plus TVs plus CABG, showing a significant overprediction of mortality by the EuroSCORE II (O/E ratio 0.40; 95% CI: -0.15–0.95) (Table 3). The EuroSCORE II discriminative power was acceptable (AUCs = 0.72) for the low-risk and high-risk groups while it failed to confirm a good discrimination in the moderate-risk group as well as in all subcategories of high-risk group (Table 4). The H-L statistics failed to confirm a good calibration only for the high-risk group. The O/E mortality ratio confirmed a good calibration for the moderate-risk group and for all subcategories of high-risk group. On the contrary, for the high-risk category, the O/E mortality ratio showed a significant overprediction of mortality (O/E mortality ratio = 1.24; 95% CI: 1.08–1.40). However, further analysis of high risk group subcategories confirmed good calibration for all subcategories (O/E mortality ratio and H-L statistics) (Table 4). For the low-risk group, the O/E mortality ratio showed a significant underestimation of mortality (O/E mortality ratio = 0.66; 95% CI: 0.48–0.84).

Discussion

The significant progress in the development of risk prediction models in cardiac surgery was made in the last two decades. Therefore, a risk- adjusted perioperative mortality rate following cardiac surgery was widely used as an indicator for a quality of care as well as for comparison of outcomes among institutions and surgeons. Predicted probability of occurrence of postoperative death also enabled stratification of patients into the different clinical risk groups (low, moderate, high), and, subsequently, made it possible to target the high-risk surgical patients in need of new therapeutic interventions [11]. We have to point out that there is no ideal cardiac surgical risk prediction score model available. Although the Society of Thoracic Surgeons Predicted the Risk of Mortality (STS PROM) score (including 40 variables) and the EuroSCORE II (including 17 variables) were integrated into clinical guidelines [1], they are still missing some risk factors claimed to significantly contribute to perioperative mortality in cardiac surgery (preoperative anemia [12, 13], liver dysfunction [14, 15], and frailty [16, 17]).

Statistical explanation for omission of these risk factors might be that factors with a low prevalence, even if associated with a high odds ratio for the outcome (in this case – perioperative mortality) at the univariate analysis, are generally excluded by the multivariable logistic regression models [12]. Although aged, the EuroSCORE models (additive and logistic) retained a good discriminative power over the time; they failed to maintain good calibration due to an overestimation of the adult cardiac surgical risk by two-to threefold [2, 3, 18]. Therefore, they were updated and renewed into the EuroSCORE II (EuroSCORE Pilot Study, 2010). The internal testing of EuroSCORE II performances on the validation dataset (5,553 patients) confirmed a good discrimination [2] and a good calibration, too (H-L test, $p = 0.09$) [19]. The EuroSCORE II performances underwent an external validation in numerous studies, later on. Grant et al. [20] presented a validation of EuroSCORE II in a sample of 23,740 patients and supported the use of EuroSCORE II as a generic risk model for the United Kingdom contemporary cardiac surgery. Garcia-Valentin et al. [21] in their prospective, multicentre study (20 centers, 4,034 patients) concluded that the EuroSCORE II was the best model in Spain at that moment.

Table 1

Patients characteristics and operative details for the study population compared with the original EuroSCORE II dataset

Variable	EuroSCORE II		*p*-value
	(our database, n = 10,048)	(original database n = 22,381)*	
Age (years), mean ± SD	63.2 ± 10.5	64.6 ± 12.5	0.0001
Gender (female), n (%)	2,963 (29.5)	6,919 (30.9)	0.01
Renal impairment, n (%)			
normal	5,218 (51.9)		
moderate	3,826 (38.1)		
severe	951 (9.5)		
dialysis	53 (0.5)	244 (1.1)	0.0001
Extracardiac arteriopaty, n (%)	1,769 (17.6)		
Poor mobility, n (%)	71 (0.7)		
Previous cardiac surgery, n (%)	359 (3.6)		
Chronic lung disease, n (%)	529 (5.3)	2,384 (10.7)	0.0001
Active endocarditis, n (%)	122 (1.2)	497 (2.2)	0.0001
Critical preoperative care, n (%)	91 (0.9)	924 (4.1)	0.0001
Diabetic on insulin, n (%)	1,028 (10.2)	1,705 (7.6)	0.0001
NYHA Class, n (%)			
I	1,331 (13.2)		
II	6,141 (61.1)		
III	2,440 (24.3)		
IV	136 (1.4)		
CCS Class IV, n (%)	797 (7.9)		
Left ventricle function, n (%)			
good	3,900 (38.8)		
moderate	4,632 (46.1)		
poor	965 (9.6)		
very poor	551 (5.5)		
Recent myocardial infarction, n (%)	1,209 (12.0)		
Pulmonary hypertension, n (%)			
moderate	2,932 (29.2)		
severe	881 (8.8)		
Urgency, n (%)			
elective	7,590 (75.5)	17,165 (76.7)	0.02
urgent	1,763 (17.5)	4,135 (18.5)	0.04
emergency	684 (6.1)	972 (4.3)	0.0001
salvage	11 (0.1)	109 (0.5)	0.0001
Weight of the intervention, n (%)			
isolated CABG	5,228 (52.0)	10,448 (46.7)	0.0001
single non-CABG	2,002 (19.9)		
two procedures	2,007 (20.0)		
three procedures	811 (8.1)		
Surgery on thoracic aorta, n (%)	747 (7.4)	1,636 (7.3)	0.70
EuroSCORE II (%)	3. 61	3. 90	

***Data available from the original manuscript by Nashef et al. [2]; EuroSCORE – European System for Cardiac Operative Risk Evaluation; NYHA – New York Heart Association; CCS – Canadian Cardiovascular Society; CABG – coronary artery bypass grafting.**

n (%) – number (percentage) of patients.

Table 2

Validation of EuroSCORE II performances in Serbian cardiovascular centers

Center Cardiac procedures (number of patients)	Mortality, n (%)		O/E ratio	(95% CI)	H-L *p*-value	AUC (95% CI)
	observed	expected				
ICD Vojvodina [5]						
All patients (n = 1,247)	43 (3.45)	27 (2.13)	1.59	(1.12–.06)	0.14	0.74 (0.67–0.82)
CABG (n = 718)	16 (2.23)	12 (1.67)	1.33	(0.68–1.98)	0.035	0.72 (0.58–0.86)
Valve(s) (n = 294)	11 (3.74)	6 (2.00)	1.83	(0.7–2.91)	0.49	0.73 (0.57–0.89)
Combined (n = 233)	16 (6.87)	9 (3.65)	1.78	(0.91 –2.65)	0.64	0.68 (0.53–0.82)
Aortic /						
Other CP /						
ICD Dedinje [6], (one-year data)						
All patients (n = 1,864)	68 (3.65)	65 (3.51)	1.05	(0.81–1.29)	0.003	0.85 (0.81–0.89)
CABG (n = 1,039)	24 (2.31)	25 (2.39)	0.96	(0.58–1.34)	0.038	0.81 (0.72–0.91)
Valve(s) (n = 410)	15 (3.66)	14 (3.42)	1.07	(0.5–1.61)	0.26	0.91 (0.86–0.96)
Combined (n = 260)	13 (5.00)	16 (6.16)	0.81	(0.37–1.25)	0.52	0.72 (0.58–0.86)
Aortic (n = 122)	16 (13.1)	10 (8.00)	1.60	(0.82–2.38)	0.28	0.82 (0.74–0.91)
Other CP (n = 33)	0 (0.00)	1 (2.47)	N/A	N/A	N/A	N/A
ICD Dedinje > 5 year data,						
(> 10,000 patients)						
All patients (n = 10,048)	388 (3.86)	363 (3.61)	1.07	(0.96 – 1.18)	0.0001	0.84 (0.82–0.86)
CABG (n = 5,228)	117 (2.24)	126 (2.41)	0.93	(0.76 – 1.10)	0.0001	0.84 (0.80–0.87)
Valve(s) (n = 2,305)	69 (2.99)	71 (3.10)	0.97	(0.74 – 1.20)	0.006	0.86 (0.81–0.90)
Combined (n = 1,569)	99 (6.31)	102 (6.51)	0.97	(0.78 – 1.26)	0.001	0.78 (0.73–0.83)
Aortic (n = 747)	95 (12.7)	58 (7.82)	1.64	(1.31 – 1.97)	0.005	0.76 (0.70–0.81)
Other CP (n = 199)	8 (4.02)	5 (2.60)	1.60	(0.49 – 2.71)	0.61	0.79 (0.61–0.98)

EuroSCORE – European System for Cardiac Operative Risk Evaluation; ICD – Institute for Cardiovascular Diseases; N/A – not applicable; CP – cardiac procedures; CABG – coronary artery bypass grafting; O/E – observed to expected; H-L – Hosmer-Lemeshow; AUC – area under the receiver operating characteristic curve.

Table 3

Calibration and discrimination of EuroSCORE II across more specific procedures [valve(s) and combined surgery]

Type of surgery	Patients (n)	Observed mortality n (%)	Expected mortality n (%)	O / E ratio	(95% CI)	H-L *p*-value	AUC (95% CI)
AVR	(979)	21 (2.15)	24 (2.44)	0.88	(0.51–1.25)	0.18	0.86 (0.78–0.93)
AVR + CABG	(835)	44 (5.27)	46 (5.51)	0.96	(0.68–1.24)	0.55	0.74 (0.66–0.82)
MVR	(399)	11 (2.76)	12 (2.96)	0.92	(0.38–1.46)	0.44	0.81 (0.70–0.92)
MVR + CABG	(217)	20 (9.22)	16 (7.18)	1.25	(0.70–1.80)	0.14	0.82 (0.72–0.92)
MVR + TVs	(157)	7 (4.46)	8 (5.11)	0.88	(0.23–1.53)	0.37	0.64 (0.40–0.88)
MVR + TVs + CABG	(45)	2 (4.44)	5 (11.05)	0.40	(-0.15–0.95)	0.99	0.95 (0.00–1.00)
MVr	(437)	3 (0.69)	6 (1.46)	0.50	(-0.07–1.07)	0.51	0.70 (0.00–1.00)
MVr + CABG	(286)	13 (4.55)	16 (5.51)	0.81	(0.37–1.25)	0.23	0.85 (0.37–1.25)
MVr + TVs	(85)	5 (5.88)	3 (3.53)	1.67	(0.21–3.13)	0.80	0.83 (0.68–0.98)
MVr + TVs + CABG	(45)	3 (6.67)	5 (10.81)	0.60	(-0.08–1.28)	0.36	0.45 (0.00–0.92)
AVR + MVR	(157)	14 (8.92)	11 (7.10)	1.27	(0.60–1.94)	0.49	0.84 (0.73–0.96)
AVR + MVR + CABG	(64)	8 (12.5)	7 (10.7)	1.14	(0.35–1.93)	0.38	0.88 (0.00–1.00)
AVR + MVr	(91)	8 (8.79)	7 (8.02)	1.14	(0.35–1.93)	0.34	0.89 (0.78–1.00)
AVR + MVr + CABG	(77)	9 (11.7)	8 (10.44)	1.13	(0.40–1.86)	0.66	0.78 (0.59–0.96)

EuroSCORE – European System for Cardiac Operative Risk Evaluation; AVR – aortic valve replacement; MVR – mitral valve replacement; MVr – mitral valve reconstruction; TVs – tricuspid valve surgery; CABG – coronary artery bypass grafting; O/E – observed to expected; CI – confidence interval; H-L – Hosmer-Lemeshow; AUC – area under the receiver operating characteristic curve; n – number of patients.

Table 4

Calibration and discrimination of EuroSCORE II across arbitrary determined risk group categories

EuroSCORE II risk group (predicted risk %)	Mortality, n (%)		O/E ratio	(95% CI)	H-L *p*-value	AUC (95% CI)
	observed	expected				
Low (0.5–2.5) [6,000 (59.7 %)]	52 (0.87)	79 (1.32)	0.66	(0.48–0.84)	0.81	0.72 (0.65–0.79)
Moderate (> 2.5–6.5) [2,730 (27.2%)]	118 (4.32)	108 (3.96)	1.09	(0.89–1.29)	0.18	0.64 (0.58–0.69)
High (> 6.5) [1,318 (13.1%)]	218 (16.5)	176 (13.4)	1.24	(1.08–1.40)	0.007	0.72 (0.68–0.75)
Higher (> 6.5–13.5) [923 (9.18%)]	103 (11.16)	84 (9.08)	1.23	(0.99–1.47)	0.78	0.67 (0.61–0.759
Very high (> 13.5–20.0) [216 (2.14%)]	46 (21.3)	35 (16.3)	1.31	(0.93–1.69)	0.22	0.51 (041–0.60)
Extremely high (> 20.0) [179 (1.78%)]	69 (38.55)	57 (31.8)	1.21	(0.92–1.50)	0.38	0.68 (0.60–0.76)

EuroSCORE – European System for Cardiac Operative Risk Evaluation; O/E – observed to expected; CI – confidence interval; H-L – Hosmer-Lemeshow; AUC – area under the receiver operating characteristic curve.

In a meta-analysis [22] of 22 studies involving 145,592 cardiac surgery procedures, the authors concluded that the EuroSCORE II showed good overall performances in terms of discrimination and accuracy of model prediction for operative mortality in cardiac surgery. Although four cardiac centers from Serbia [2] contributed to the EuroSCORE II development dataset with more than 1,000 patients (≈ 5% of database), it would be reasonable to expect that the EuroSCORE II would also be an appropriate model for prediction of operative mortality in Serbian patients undergoing open heart surgery. Indeed, the initial results of EuroSCORE II validation in the Serbian cardiac surgical cohort [5, 6], confirmed an overall good discriminative power. Calibration using the O/E mortality ratio was good in all categories, excluding a significant underprediction of mortality (O/E ratio 1.59; 95% CI 1.12–2.06) for the category 'all patients' of ICD Vojvodina sample [5] (Table 2).

The analysis of our 5-year results confirmed a very good discriminative power of the EuroSCORE II for the whole cohort (AUC = 0.84) as well as for all subgroups of performed cardiac procedures (AUCs from 0.76 to 0.86) (Table 2). The H-L statistics confirmed a good calibration only for the subgroup 'other cardiac procedures'. Although we tested a huge sample, it was not a big surprise that the H-L goodness of fit test did not perform well. Namely, in order to achieve proper conditions to obtain more precise calibration, Paul et al. [23] requested that the huge samples should be divided into more groups (> 2,000 patients in 34, and > 4,000 patients in 130 groups), which was impossible to perform using the statistical package SPSS version 17.0. On the contrary, the O/E mortality ratio (including 95% CI values) confirmed a good calibration for all categories, except for the 'aortic surgery' (significant underestimation of mortality, O/E ratio 1.64; 95% CI: 1.31–1.97). Although O/E mortality ratio for aortic surgery was very close to our previously published [6] result (1.64 vs. 1.60) with a larger sample, the difference (showing underestimation of mortality) became statistically significant. So far, a very few authors have reported results on the thoracic aorta surgery using the EuroSCORE II prediction of mortality. Chalmers et al. [24] reported in-hospital mortality of 6.8% with the median EuroSCORE II value of 5.6% (interquartile range 3.1% to 11.1%). Nishida et al. [25] presented 461 consecutive patients undergoing thoracic aorta surgery with the observed mortality of 7.2%, with the average EuroSCORE II value of 7.4%. We have to point out the possibility that, generally speaking, the overestimation of mortality risk by the risk stratification model may result from publication bias, namely, studies which obtained favorable results could be reported more easily, while authors with unfavorable results (significantly worse outcome compared with predicted mortality) are not so willing to publish their results [22, 26, 27]. Therefore, we have checked our results in the elective and urgent/emergent aortic surgery. In the elective aortic surgery [mortality 5.07% (22 out of 434)], a discriminative power of EuroSCORE II was acceptable (AUC = 0.702), while calibration showed a nonsignificant overprediction of mortality (expected mortality of 6.49%; O/E ratio 0.79; 95% CI 0.46–1.12) by the EuroSCORE II. Thus, for the elective aortic surgery, the EuroSCORE II confirmed a good discrimination and calibration. In the urgent/emergent aortic surgery [mortality 23.3% (73 out of 313)] a discriminative power of the EuroSCORE II was good (AUC = 0.74), while calibration showed a significant underprediction of mortality (expected mortality of 9.7%; O/E ratio 2.43; 95% CI 1.87–2.99) by the EuroSCORE II. However, in a real world scenario, in the patients undergoing aortic surgery for the acute aortic dissection, early mortality still remains high, ranging from 17% to 26% [28–32]. Underprediction of operative mortality by the EuroSCORE II in this category might be attributed to the fact that some very important risk factors are not included in the EuroSCORE II variables [neurological dysfunction/comma, organ system malperfusion (especially visceral/mesenteric ischemia/infarction), hypotension, possible cardiac tamponade, ongoing cardiac ischemia, etc] [29, 30, 32]. Currently, the observations from the German Registry for the Acute Aortic Dissection Type A (GERAADA) (50 cardiac surgery centers in Austria, Switzerland and Germany, including 2,137 patients), confirm a progressively escalating

mortality with each additional malperfused organ system (adjusted odds ratio for one organ = 1.65, two organs = 2.44, three, or more = 3.39; $p < 0.0001$) [32].

The overall observed mortality for our whole cohort showed a slight, nonsignificant underprediction of mortality (O/E ratio 1.07; 95% CI 0.96–1.18). Several studies, including thousands of patients, confirmed that the EuroSCORE II significantly overpredict mortality [Guida et al. [22] (145,592 patients), O/E = 0.89, 95% CI 0.86–0.92; Grant et al. [20] (23,740 patients), O/E = 0.91, 95% CI 0.84–0.98; Osnabrugge et al. [33] [21,016 patients, operated after 1st January 2008; it is a part of a whole cohort (50,558 patients) which represent more contemporary sample, and it would be used in further comparisons, O/E = 0.80, 95% CI 0.73–0.87]. On the other hand, several studies confirmed a significant underprediction of mortality by the EuroSCORE II [Velicki et al. [5] (1,247 patients), O/E = 1.59, 95% CI 1.12–2.06; Arnaiz-Garcia et al. [34] (1,200 patients), O/E = 1.86, 95% CI 1.46–2.26; Chalmers et al. [24] (5,576 patients), O/E = 1.71, 95% CI 1.47–1.95 [35]]. In the CABG, valve(s) and combined surgery in our cohort, the observed mortality was slightly (but not statistically significantly) better than the predicted by the EuroSCORE II (Table 2). The significantly better results (compared with predicted mortality by EuroSCORE II) in CABG surgery were reported by Grant et al. [20] (12,470 patients), O/E = 0.71, 95% CI 0.61–0.81; as well as by Osnabrugge et al. [33] (16,096 patients), O/E = 0.77, 95% CI 0.74–0.80]. On the contrary, Kunt et al. [18] (428 CABG patients over 70 years of age) presented extremely poor prediction, with an O/E ratio of 4.86, 95% CI 3.03–6.43. To the best of our knowledge, the EuroSCORE II validation of more specific procedures (AVR, MVR, MVr, multiple valve surgery, and combined procedures – valve(s) surgery with CABG surgery), have not yet been presented for the Serbian cardiac surgical population. For AVR and AVR plus CABG surgery, we performed slightly (not significantly) better than predicted by the EuroSCORE II (O/E mortality ratio of 0.88 and 0.96, respectively). Those results coincides with large series by Osnabrugge et al. [33] (2,170 AVR patients, and 1,627 AVR plus CABG cases, O/E ratio of 1.14, 95% CI 0.87–1.40 and 0.76, 95% CI 0.58–0.95; respectively), and by Biancari et al. [36] including 11,791 AVR patients, with the O/E ratio of 0.94. In mitral valve reconstructive surgery, we achieved excellent result (mortality 0.69%, 3 of 437 patients) compared with the predicted mortality (1.46%) by the EuroSCORE II (O/E ratio of 0.5, 95% CI -0.07–1.07). This result is almost comparable with reference mitral valve center (Ottawa) [37] result – mortality of 0.60% (5 of 851, O/E ratio of 0.24, 95% CI 0.03–0.51). For MVr surgery, Osnabrugge et al. [33] reported 624 patients with the O/E ratio of 0.64, 95% CI 0.17–1.11. In MVR surgery, Chan et al. [37] again presented the significantly better result than predicted by the EuroSCORE II (6 of 303, O/E ratio of 0.44, 95% CI 0.07–0.81). On the contrary, the Osnabrugge's group [33] observed nonsignificant underestimation of mortality by the EuroSCORE II for MVR surgery (O/E ratio 1.34, 95% CI 0.87–1.81). We (in 399 patients) observed slightly better results than predicted (O/E ratio 0.92). For all other more specific procedures, usable data are not available in relevant literature (small samples, incomplete data, non-contemporary cohorts, etc).

The acceptable discriminative power of EuroSCORE II was detected for low-risk (AUC – 0.72) and high-risk group (AUC – 0.72) category. The EuroSCORE II failed to confirm a good discriminative power for moderate-risk category and in all high-risk group subcategories. The explanation for reduced discriminative power is statistically simple. When the patients are stratified according to the risk score, and then only one strata is analysed, the regressors and their coefficients within the stratum are different from those which allocated them to that risk group in the first place [38]. Furthermore, a minimum of 100 (and preferably 200) events (perioperative deaths) should be included in the sample size so that the model performance can be adequately assessed [39]. The HL statistics failed to confirm a good calibration only for the high-risk group category. According to the O/E mortality ratio, for the low-risk group, the model significantly overestimate mortality (O/E ratio 0.66, 95% CI 0.48–0.84), while it slightly, but significantly underestimate mortality in the high-risk group (O/E 1.24, 95% CI 1.08–1.40). On the contrary, further analysis of high-risk group subcategories confirmed a good calibration for all subcategories. Although we confirmed a good calibration in all subcategories of high-risk category, our results are not in accordance with the previous statements that the EuroSCORE II significantly underestimate mortality in the high-risk group category [5, 8, 40, 41]. Regarding the results in all high-risk group subcategories, our study is in keeping with the results of Barili et al. [3] who showed an optimal EuroSCORE II calibration until 30%-predicted mortality. The results of our study show an acceptable overall performances of the EuroSCORE II risk stratification model in terms of discrimination and accuracy of model, when applied to the Serbian contemporary cardiac surgical cohort undergoing open heart surgery at our Institute.

Limitations of the study

The limitation of our study is its single-center design, and therefore results may not represent national and international practice and outcomes. Although our cohort recruited more than 10,000 patients, another limitation was a sample size, which generated relatively small specimens, including the limited number of tested events (in this case perioperative deaths) for more precise analysis of some subgroups.

Conclusion

The results of our study confirmed acceptable overall performances of the EuroSCORE II risk stratification model in terms of discrimination and accuracy of model, when applied to the Serbian contemporary cardiac surgical cohort undergoing open heart surgery at our Institute.

Conflict of interest

None declared.

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Tuberous sclerosis complex, Serbian referral center experience

Kompleks tuberozne skleroze – kliničko iskustvo jednog referentnog centra u Srbiji

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Abstract

BackgroundAim. Common features of tuberous sclerosis complex (TSC) arise from the formation of hamartomas both in the brain and multiple organ systems, mainly due to a mutation in one of two genes, TSC1 or TSC2, with well described inter- and intrafamilial different phenotypic outcomes. The aim of this work was to make a synthesis of the patients data with diagnosed tuberous sclerosis in order to better understand the disease in our environment. **Methods.** We reviewed retrospectively the clinical records of all patients with TSC, diagnosed and regularly followed at the Clinic of Neurology and Psychiatry for Children and Youth in Belgrade, Serbia during the period of more than two decades. Statistical analyses were performed using descriptive statistics as well as the Fisher's exact test. **Results.** Cohort of 44 patients with the diagnosis of definitive TSC were included. The mean age at last follow-up was 19.4 years [age range 1–58, standard deviation (SD) 11.8]. Family history for TSC was noted in 25% of patients. Dermatological manifestations were described in 93.2%, retinal astrocytoma and cardiac rhabdomyomas was found in 36.4% each, *n*ephrological manifestations in 34.1% and lymphangioleiomyomatosis was diagnosed in two female patients. All patients presented with the structural lesions of central nervous system; epilepsy was diagnosed in 88.6%, out of whom 59 % of patients had seizure onset in the first year of life. The West syndrome was diagnosed in 27.3% of patients. Complete seizure control was achieved in 30.8%, in a majority with valproic acid or cabamazepine, but also with topiramate, lamotrigine and vigabatrin. At least two antiepileptic drugs were administered in 82% of patients. Mental retardation was noted in 50% of patients. Psychiatric manifestations were found in 40.9%, with attention deficit hyperactivity disorder diagnosed in 27.3%, autism spectrum disorder in 13.6 %, and psychosis and depression observed in 11.4% each. **Conclusion.** This kind of synthesis of the data certainly contributes to better understanding of the disease in our environment, as TSC, although well-known disease, still remains diagnostic and therapeutic challenge in daily clinical practice.

Key words:
tuberous sclerosis; epilepsy; diagnosis; drug therapy; serbia.

Apstrakt

Uvod/Cilj. Karakteristike kompleksa tuberozne skleroze uzrokovane su formiranjem hamartoma u mozgu i velikom broju organa, najčešće kao posledice mutacije u jednom od dva gena, TSC1 ili TSC2, sa veoma dobro dokumentovanom inter- i intrafamilijarnom razlikom u fenotipu. Cilj ovog rada bio je da se sintetišu podaci o bolesnicima sa dijagnostikovanom tuberoznom sklerozom radi boljeg razumevanja bolesti u našem okuženju. **Metode.** Retrospektivno je analizirana medicinska dokumentacija svih bolesnika sa kompleksom tuberozne skleroze, dijagnostikovanih i lečenih u Klinici za neurologiju i psihijatriju za decu i omladinu u Beogradu, Srbija, tokom vremenskog perioda dužeg od dve decenije. Statistička analiza sprovedena je merama deskriptivne statistike, kao i upotrebom Fišerovog testa. **Rezultati.** Analizirana je kohorta od 44 bolesnika sa dijagnozom definitivnog kompleksa tuberozne skleroze. Srednja vrednost životnog doba na poslednjem pregledu bila je 19.4 godine [uz raspon godina 1–58, standardna devijacija (SD) 11,8 godine]. Porodična pojava bolesti zabeležena je kod 25% bolesnika. Dermatološke manifestacije opisane su kod 93,2%, retinalni astrocitomi i rabdomiomi srca nađeni su kod po 36,4%, nefrološke manifestacije kod 34,1%, dok je limfangioleiomiomatoza dijagnostikovana kod dve bolesnice. Svi bolesnici su imali strukturne lezije centralnog nervnog sistema; epilepsija je dijagnostikovana kod 88,6% bolesnika, od kojih se kod 59% bolesnika prvi napad javio u prvoj godini života. Westov sindrom dijagnostikovan je kod 27,3% bolesnika. Potpuna kontrola epileptičkih napada postignuta je kod 30,8% bolesnika, u većini upotrebom valproične kiseline ili karbamazepina, ali i topiramata, lamotrigina i vigabatrina. Kod 82% bolesnika primenjena su najmanje dva antiepileptika. Mentalna retardacija je utvrđena

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kod 50% bolesnika. Psihijatrijske manifestacije bolesti bile su zapažene kod 40,9% bolesnika, od čega je poremećaj pažnje i hiperaktivnosti dijagnostikovan kod 27,3%, spektar autističnih poremećaja kod 13,6%, psihoza i depresija kod po 11,4% bolesnika. **Zaključak.** Sačinjena sinteza podataka doprinosi boljem razumevanju bolesti u našem okruženju, jer kompleks tuberozne skleroze, iako dobro definisano oboljenje, i dalje predstavlja dijagnostički i terapijski izazov u svakodnevnoj kliničkoj praksi.

Ključne reči:
skleroza, tuberozna; epilepsija; dijagnoza; lečenje lekovima; srbija.

Introduction

As a multisystem genetic disease with variable expression, tuberous sclerosis complex (TSC) represents a challenge for both diagnosing and management of different aspects of the disease. The estimated frequency of TSC ranges from 1:6,000 to 1:10,000 live births and population prevalence is around 1 in 20,000 [1]. Approximately 85% of patients with TSC have a mutation in one of two genes TSC1 or TSC2, with described inter- and intrafamilial different phenotypic outcomes [2]. Common features of TSC arise from the formation of hamartomas in multiple organ systems. The central nervous system (CNS) implies specific brain lesions, such as cortical tubers, subependymal nodules (SEN), subependymal giant cell astrocytomas (SEGA) and heterotopic bands in the white matter, clinically often manifested as intractable epilepsy, mental retardation, autistic spectrum disorder, psychosis and behavioral disorders. Cortical tubers are seen as the primary site of epileptogenesis, even though non tuber regions of cortex may be capable of generating seizures, that present a challenge in surgical treatment of epilepsy in TSC [3]. Early onset of seizures, during the first year of life, carries a high risk of neurodevelopmental and cognitive impairments. The seizure control must be accomplished as early as possible in the course of the disease in order to achieve more favorable outcome of condition [4–6]. Recommendations for the treatment of epilepsy in the patients with TSC are given at an international TSC consensus conference in Rome 2012 [7]. The latest clinical controlled study reflects the favorable effect of adjunctive everolimus therapy for pharmacoresistant focal epilepsy associated with TSC [8]. Characteristic multisystem involvement in TSC includes the skin lesions, kidney and lung lesions, retinal hamartomas and heart rhabdomyomas, that all contribute significantly to the severity of clinical presentation of disease. The individuals with TSC also have a range of behavioral, psychiatric, intellectual, academic, neuropsychologic and psychosocial difficulties. Their importance has become more visible by introducing a new term – TSC associated neuropsychiatric disorders (TAND), which can be assessed using a specific checklist [9].

Methods

We reviewed retrospectively the clinical records of all patients with TSC, diagnosed and regularly followed at the Clinic of Neurology and Psychiatry for Children and Youth in Belgrade, Serbia, during the period of more than two decades (1993–2015). The patients were included if they met the International tuberous sclerosis diagnostic clinical criteria for definite TSC [1]. Charts were reviewed for the history of multisystem visceral involvement, morphological CNS manifestations and epilepsy, including age of onset, occurrence and frequency of multiple seizure types, level of the seizure control, response to antiepileptic non-pharmacological therapy and/or antiepileptic drugs (AEDs). Intellectual impairment was seen through the intelligence quotient (IQ), and where IQ was not obtained (in 7 patients), a developmental quotient (DQ) was considered equal parameter of intellectual capacity. Patients with an IQ or DQ lower than 70 were considered to be the subjects with mental insufficiency. Psychiatric pathologies, such as, behavior and mood disorders, autism spectrum disorder (ASD) and psychosis, were evaluated and diagnosed by psychiatrist. We also assessed the relationship between the neurological and psychiatric manifestations of TSC, such as seizure control in certain psychiatric disorders.

Statistical analyses were performed using descriptive statistics (absolute and percentage values with the determination of the arithmetic mean and standard deviation) as well as the Fisher's exact test.

Results

A cohort of 44 patients with the diagnosis of definitive TSC were included, among them 26 were females, and 18 males. The mean age at last follow-up was 19.4 years [age range 1–58, standard devation – SD 11.8). The family history for TSC was noted in 11 (25%) patients.

The mutational analysis was available for 10 (6 females, 4 males) patients only. The results of study disclosed the TSC1 mutation in 3, the TSC2 mutation in 5 and no mutation in 2 patients. The testing using the multiplex ligation-dependent probe amplification (MLPA), or other method was advised to exclude the presence of possibly pathogenic deletions of single or multiple exons.

All patients presented with the structural lesions of central nervous system, among them 93.2% had subependymal nodules and 81.8% had cortical tubers. The subependymal giant cell astrocytoma (SEGA) was diagnosed in 15 (34.1%) patients and it was successfully operated in 10 patients, but one female patient who was reoperated at the age of 4 years due to tumor rest recurrence. Dermatological manifestations were described in 41 (93.2%) patients; hypomelanotic macules were present in 81.8%, facial angiofibromas in 68.2%, shagreen patch was observed in 31.8%, hyperpigmentations in 22.7% and other types of skin lesions in 25% of patients. Retinal astrocytoma and cardiac rhabdomyomas was found in 36.4% each. Nephrological manifestations were present in

34.1%, among them 27.3% had renal cysts and 53.3% renal angiomyolipoma (AML). Two patients with multiple AML were nephrectomised. Lymphangioleiomyomatosis (LAM) was diagnosed in 2 female patients, after the age of 20 and 40 years.

Focal neurological deficit was described in 36.4% and slowed early psychomotor development was seen in 22.7%. Mental retardation was present in 50% of patients, with the following distribution of 27.2% having mild, 22.7% moderate, 9.1 % severe, 13.6% profound mental retardation and remaining 27.2% of patient were without accurate level of mental retardation.

Epilepsy was diagnosed in 39 (88.6%) patients with TSC. The epileptic seizures were revealing clinical manifestation of the TSC in almost all patients with the TSC and epilepsy, but 2 in whom the first manifestation was congenital rhabdomyoma. In the remaining 5 patients without epilepsy, the first manifestations of the disease were: in a 3-year old girl a headache and double vision as a sign of increased intracranial pressure, indicating SEGA; in a 13-years old girl ungual fibroma; in a 15-years old boy weight loss and leg pain, when the skin lesions were noticed; in a 15-years old girl an acute psychosis with productive symptoms and in the fifth female patient skin lesions in adulthood, after the diagnosis of TSC was established in her daughter. The mean age of the first seizure onset was 2.8 years (range from 1 month to 16 years, SD 4.1), with 59% of patients with seizure onset in the first year of life. The West syndrome was diagnosed in 27.3% of patients. In 16.7% of patients with the West syndrome, the focal seizure preceded the occurrence of infantile spasms. Of 12 patients with a history of West syndrome, 11 (91.7%) were cognitively impaired, compared with 11 of 27 (40.7%) patients without a history of West syndrome (p = 0.003). The focal seizures were present in 84.6% of patients with epilepsy, of which secondary generalized seizures were recorded in 39.3% of cases. Coexistence of focal seizures and infantile spasms were described in 23.1% of patient with the TSC and epilepsy. Other seizure types were tonic, atonic and absences. The complete seizure control was achieved in 30.8%, in a majority with valproic acid or cabamazepine, but also with topiramate, lamotrigine and vigabatrin. At least two antiepileptic drugs were administered in 82% of patients.

Psychiatric manifestations of TSC in our group of patients were present in 40.9%, with attention deficit hyperactivity disorder (ADHD) diagnosed in 27.3%, ASD in 13.6 %, and psychosis and depression present in 11.4% each. All patients with TSC and ADHD had epilepsy, and one third maintained the complete seizure control while on medication. One-quarter of patients with ADHD and TSC had ASD, and 75% of patients with ADHD and TSC had mental retardation. Of 6 patients with TSC and ASD, 5 had epilepsy diagnosed, of whom 3 with the complete seizure control and one with more than 75% reduction in the seizure frequency. The opposite was seen in the group of patients with TSC and psychosis, where unfavorable seizure control was present in 3 out of 4patients.

We compared two subgroups of patients with TSC and with/without SEGA and we found slight differences in some clinical characteristics, but not reaching statistical significance (Table 1). Of 15 patients with SEGA, 5 had a positive family history for TSC compared with 6 of 29 patients without SEGA. The West syndrome was diagnosed in 5 patients with SEGA and in 7 without SEGA, neurological deficits were seen in 8 patients with SEGA compared with 8 patients without SEGA. Intellectual impairment was present in 8 patients with SEGA compared with 14 patients without SEGA. We did not find a higher incidence of neuropsychiatric disorders (ASD, ADHD, psychosis, depressive disorder) in the group of patients with SEGA compared with the patients without SEGA.

Table 1
Comparison of two subgroups of patients with tuberous sclerosis complex (TSC) and with/without the subependymal giant cell astrocytoma (SEGA)

Clinical parameter	Patients with SEGA n (%)	Patients without SEGA n (%)
Family history	5 (33.3)	6 (20.7)
West syndrome	5 (33.3)	7 (21.4)
Neurological deficits	8 (53.3)	8 (27.6)
Intellectual impairment	8 (53.3)	14 (48.3)
Neuropsychiatric disorders	5 (33.3)	11 (37.9)

Discussion

A series of 44 patients with the diagnosis of definitive TSC was retrospectively assessed. Positive family history for TSC was seen in 25% of patients indicating a high level of *de novo* mutations, which is in concordance with data from the relevant literature [2]. The molecular genetic studies were conducted only in a limited number of patients by sending samples abroad, due to the lack of this analysis in the country. The frequency of mutations in TSC2 is higher than in TSC1. Our limited experience confirms this observation (5/10 of our patients have mutation in TSC2 gene). There is some evidence from case series that mutations in TSC2 tend to result in more severe disease. No mutation is identifiable in 15%–20% of TS patients and these patients generally have milder clinical manifestations [10]. Both of our patients (15 and 21 month old children) with no mutation identified had favorable seizure control and normal early psychomotor development, but the long-term clinical follow-up will be necessary.

SEGA, as a characteristic brain tumor, was present in 34.1% of our cohort, that is higher than usually described frequency of 10%–20% of patients with TSC [11]. The probable reason is partly related to the selection bias. Our institution is the regional neuropediatric reference center and often receives referrals of operated children with SEGA as early manifestation of the disease for further clinical monitoring. In our group, the dermatological manifestations were described in 93.2%, compared to up to 96% in a published series of patients [12]. Retinal astrocytoma were present in 36.4%, lower than usually described (around 50%). These benign retinal tumors tend to regress during the course of the disease and it is possible that this process occurred in our pa-

tients who were referred and diagnosed at later age. According to the data from the literature, cardiac rhabdomyomas was present in 20% to 60% of patients, depending on the age, and in our group it was seen, with cardiac ultrasound, in 36.4%. In the study of Jóźwiak et al. [13], of 154 patients, 48% of them had cardiac rhabdomyomas, the majority of which was asymptomatic. In 2 our patients, the congenital cardiac rhabdomyoma was clinical TSC manifestation. In one of them, it was removed during the first year of life. Similar results were found in Osaka where percentage of registered cardiac rhabdomyomas was 49% [14]. The nephrological manifestations were present in 34.1% of our patients, among them 53.3% had AML. This is lower than expected (up to 80%) and one of the reasons may be the incomplete medical reports done in other institutions during the outpatient follow-up. LAM was diagnosed in 2 female patient only, although this manifestation is present in 40% of patients with TSC. LAM is related to older age and our patient mainly belonged to the population of children, youth and young adults. Only two of our patients with LAM were females and diagnosed at the age of 20 and 41 years. Johnson et al. [15], describing the characteristics of the disease and its progression in 57 female patients, stated 33.6 years as the mean age of onset of the first symptoms of LAM.

Intellectual impairment was present in 50% of our patients. Early studies described more frequent occurrence of intellectual impairment (60% and above), but the new studies state the frequency of 42% to 44%, noting the association of early seizure onset, presence of mixed seizure type and lower intellectual capacity [16, 17].

The most common neurological manifestations of TSC was epilepsy, diagnosed in 88.6% of our patients which is a little higher than usually specified (85%) and the one of the reasons may be found in the most common indication (epilepsy) under which the patients were sent to our institution, making the sample highly selected on referral. The epileptic seizures are certainly most common first manifestation of the disease, however the first symptoms highly depend on the age at which the diagnosis is made (e.g., prenatal period - heart rhabdomyomas; adulthood - known family history) [18]. Other characteristics of our patients were similar to the large retrospective study conducted by Chu-Shore et al. [19], that included 291 patients with TSC. They described 63.2% of patients with the seizure onset in the first year of life; 37% with the history of infantile spasms (IS) and epilepsy in remission in 33.5% of patients with TSC. The patients with the West syndrome showed a greater degree of cognitive impairment compared with the patients without history of IS, as it was seen in our group of patients. Mental retardation was observed in 76% of patients with IS and TSC [4], and early recognition and aggressive treatment of IS (especially with early given vigabatrin, in the first week after the IS appearance) may be of a great importance for a favorable outcome. The focal seizures may precede, coexist or emerge after the occurrence of IS in the patients with TSC. Some authors recommended the preventive use of vigabatrin in children with TS (e.g., cardiac rhabdomyoma) without spasms, but with the EEG abnormalities. They stated that such approach could be beneficial [13]. We have not such clinical experience.

The most common psychiatric manifestation of the disease in our cohort was ADHD, diagnosed in 27.3% of patients, reaching the lower limit of indicated frequency of 30%–60% in the literature [20]. Described association between ADHD and ASD in the patients with TSC in the literature, was also seen in our group (25%), as well as considerably high number of patients with ADHD and TSC that had developed mental retardation (75%). It was demonstrated that 60% of patients with mental retardation and TSC had AHDH diagnosed [20]. Proportion of ASD in the general population is about 1%, while in the patients with TSC, this disorder is diagnosed more often, in 25%–50%. ASD in our cohort was described in 6 patients (13.6%), that is considerably higher than in the general population. Some children in our TSC group with soft and rare autistic traits were not diagnosed as ASD. In a review article, written by Curatolo et al. [21], a large number of studies on autism and related disorders in TSC was specified, stating a wide range of incidence of autism from 5% to 61%, but still emphasizing the personal experiences in their series of patients, estimating the percentage of autism at 26% in the patients with TSC. The different diagnostic tools for ASD were used with influence on the incidence of this condition. The risk factors for the development of ASD in TSC are localization of cortical tubers, malformation of cortical development, EEG localization related abnormality, epilepsy and "abnormal gene program". Although it is stated that ASD in TSC is often associated with the occurrence of pharmacoresistant epilepsy and epileptic encephalopathies, in our series of patients, this association was not observed. Of 5 patients with epilepsy and ASD, 3 had the stable, long-term, complete seizure control and one more than 75% reduction in the seizure frequency. Our patients with the seizure freedom mainly showed EEG low-amplitude, irregular, slow background activity. Occasional spike-wave discharges were noted in only one girl with ASD, TSC and diabetes mellitus as comorbid condition. However, in conclusion, we should remain restrained due to a small number of patients that had been analyzed. Disabling association of intractable seizures and ASD was mainly found in the TSC2 patients [22]. Without molecular genetic study of all our patients we are not able to comment that finding in the literature.

Some authors present the data showing the association between SEGA and ASD in the patients with TSC, that was not the case in our study group, but not reporting any significant common occurrence of SEGAs and ADHD, or depressive disorder, the same observation as it was seen in our cohort [23].

Since 2016, we prospectively started with administration of the tuberous sclerosis associated neuropsychiatric disorders (TAND) checklist [9] in all our patients with TSC. Standardized questionnaire in Serbian language will be used to better asses to the long-term mental development of patients.

Potential limitations ot the study include the retrospective model disabling verification of longitudinal data that could be helpful to allow more precise prognostic indicators

and a relatively small size of cohort which could be insufficient to achieve results with greater power.

However this kind of synthesis of the data certainly contributes to better understanding of the disease in our environment, as tuberous sclerosis complex, although well-known disease, still remains diagnostic and therapeutic challenge in daily clinical practice.

Conclusion

The characteristics and frequencies of manifestations of the disease are shown in a series of 44 patients with the diagnosis of tuberous sclerosis complex.

Family history for TSC was noted in 25% of patients. Dermatological manifestations were described in 93.2%, retinal astrocytoma and *cardiac* rhabdomyomas was found in 36.4% each, nephrological manifestations in 34.1% and LAM was diagnosed in two female patients. All patients presented with the structural lesions of central nervous system; epilepsy was diagnosed in 88.6%, out of whom 59 % of patients had seizure onset in the first year of life. Psychiatric manifestations of TSC were present in 40.9%, with ADHD diagnosed in 27.3%, ASD in 13.6 %, and psychosis and depression present in 11.4% each.

Presented data will help to better underbending of TSC in our enviroment.

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Intraocular pressure control after trabeculectomy in the patients with primary open angle glaucoma and pseudoexfoliative glaucoma followed up for 3 to 5 years

Kontrola intraokularnog pritiska kod bolesnika sa primarnim glaukomom otvorenog ugla i pseudoeksfolijativnim glaukomom tokom perioda od 3 do 5 godina nakon trabekulektomije

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Abstract

Background/Aim. Trabeculectomy is a safe procedure which effectively reduces the intraocular pressure (IOP). IOP is the most frequent indicator of success after glaucoma surgery. The aim of this work was to evaluate the long-term pressure control in primary open-angle glaucoma (POAG) and in pseudoexfoliative glaucoma (XFG) after primary trabeculectomy without the use of mitomycin-C (MMC), 3 to 5 years after trabeculectomy. **Methods.** This study involved a retrospective evaluation of 332 consecutive patients (352 eyes), 174 patients (188 eyes) with POAG (mean age of 64.0 ± 8.6 years) and 158 patients (164 eyes) with XFG (mean age of 70.7 ± 8.9 years) who underwent primary trabeculectomy between January 2007 and December 2009 at the Clinic for Eye Diseases, Clinical Center of Serbia in Belgrade. A successful control of IOP was defined as achieving IOP ≤ 21 mmHg without medication (complete success), or with a single topical medication (qualified success). **Results.** According to the type of glaucoma POAG/XFG preoperative IOP was 28.4 ± 6.3/30.4 ± 8.4 mmHg, respectively (p = 0.311) and last postoperative IOP was 16.9 ± 5.2/18.7 ± 5.9 mmHg, respectively (p = 0.681). According to the Kaplan-Meier survival curve, the complete success in the group with POAG in 1, 3 and 5 years were 85%, 75% and 58% and in the group with XFG were 82%, 70% and 56%, respectively. There was no statistically significant difference in the complete success rates between the patients with POAG and XFG. **Conclusion.** The primary goal of surgery was to achieve a sufficiently low IOP without additional medication, thus preventing progression of glaucomatous damage. In our study, the complete success in the group with POAG was achieved in 75% and 58% of the patientsin the period of 3 and 5 years after surgery, respectively and in the group with XFG complete success was achieved in 70% and 56% of the patients respectively.

Key words:
glaucoma open angle; exfoliation syndrome; trabeculectomy; intraocular pressure; treatment outcome.

Apstrakt

Uvod/Cilj. Trabekulektomija je sigurna procedura efikasnog snižavanja intraokularnog pritiska (IOP). IOP se uzima kao najčešće merilo uspeha nakon operacije glaukoma. Cilj rada je bio da se ispita dugoročan ishod nakon primarne trabekulektomije bez korišćenja antimetabolita uzimajući u obzir IOP kod bolesnika sa primarnim glaukomom otvorenog ugla i pseudoeksfolijativnim glaukomom u periodu od 3–5 godina nakon trabekulektomije. **Metode.** U studiji je retrospektivno praćeno 332 bolesnika (352 očiju), 174 bolesnika (188 očiju) sa primarnim glaukomom otvorenog ugla (prosečne starosti 64,0 ± 8,6 godina) i 158 bolesnika (164 očiju) sa pseudoeksfolijativnim glaukomom (prosečne starosti 70,7 ± 8,9 godina) kojima je izvršena trabekulektomija u periodu od januara 2007. do decembra 2009. godine na Odeljenju za glaukom Klinike za očne bolesti u Beogradu. Uspešna kontrola IOP je definisana postizanjem IOP manjim ili jednakim 21 mmHg, bez medikamentne antiglaukomne terapije (kompletan uspeh), ili sa jednom vrstom lokalne terapije (delimičan uspeh). **Rezultati.** Kod bolesnika sa primarnim glaukomom otvorenog ugla i sa pseudoeksfolijativnim glaukomom preoperativni IOP je bio 28,4 ± 6,3/30,4 ± 8,4 mmHg (p = 0,311), a postoperativni IOP

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16,9 ± 5,2/18,7 ± 5,9 mmHg (p = 0,681). Na osnovu Kaplan-Meier-ove krive preživljavanja, kompletan uspeh kod bolesnika sa primarnim glaukomom otvorenog ugla nakon 1, 3 i 5 godina 85%, 75% i 58% s kod bolesnika sa pseudoeksfolijativnim glaukomom bio je 82%, 70% i 56%. Među posmatranim grupama nije bilo statistički značajne razlike. **Zaključak.** Primarni cilj operacije bio je postizanje dovoljno niskog intraokularnog pritiska bez dodatne terapije čime bi se sprečila progresija glaukomnog oštećenja. U našoj studiji kompletan uspeh u grupi bolesnika sa primarnim glaukomom otvorenog ugla postignut je u 75% i 58% bolesnika nakon 3, odnosno pet godina, dok je u grupi bolesnika sa pseudoeksfolijativnim glaukomom kompletan uspeh postignut u 70% i 56% bolesnika.

Ključne reči:
glaukom otvorenog ugla; eksfolijativni sindrom; intraokularni pritisak; trabekulektomija; lečenje, ishod.

Introduction

Glaucoma is the second leading cause of blindness world-wide and the first cause of irreversible blindness [1]. Glaucoma treatments are directed at reducing the intraocular pressure (IOP), either pharmacologically or surgically [2, 3].

Since the first description of trabeculectomy in 1968 by Cairns [4], it has become the most widely used intervention for the treatment of glaucoma and it is still regarded as the gold standard [5, 6].

Trabeculectomy is a surgical procedure which effectively reduces the intraocular pressure (IOP) in most patients in short term. Over time, the effect of trabeculectomy decreases in a large proportion of patients so that over 3 to 5 years many need additional medical or surgical IOP control. Though morphological optic nerve changes along with visual field the alterations are much more relevant for the follow-up of disease progression, IOP is still the most frequent indicator of purely surgical success of glaucoma surgery.

Prior studies described the long-term effects of trabeculectomy on the IOP [7–11]. The definition 'long-term' is used for the follow-up period ≥ 1 year. The long-term successful control of IOP after primary trabeculectomy ranged from 48%–98%, depending on the follow-up period and the criteria used to define successful outcome [12–14].

In the majority of studies, the postoperative complete success in terms of IOP was described as IOP of 21 mmHg or less, without medication [7, 15, 16].

The current study evaluates the long-term pressure control in primary open-angle glaucoma (POAG) and in pseudoexfoliative glaucoma (XFG) after primary trabeculectomy without the use of mitomycin-C (MMC), 3 to 5 years after trabeculectomy.

Methods

This study involved the retrospective evaluation of 332 consecutive patients (352 eyes); 174 patients (188 eyes) with POAG (mean age of 64.0 ± 8.6 years) and 158 patients (164 eyes) with XFG (mean age of 70.7 ± 8.9 years), who underwent primary trabeculectomy between January 2007 and December 2009 at the Clinic for Eye Diseases, Clinical Center of Serbia in Belgrade, and the follow-up was 3 to 5 years after surgery.

The patients were included in this study based on the following criteria: (1) age > 40 years at the time of the surgery; (2) trabeculectomies performed without antimetabolites; (3) no previous eye surgery or laser intervention; (4) patients with the follow-up period ≥ 3 years after trabeculectomy. The criteria for trabeculectomy included: (1) inability to reach target IOP with maximum tolerated medical therapy in the patients (use of 3 or more topical medications); (2) use of oral medicines (carbonic anhydrase inhibitor); (3) cases where structural and functional progression of disease occurred despite a normal range of IOP with maximum tolerated medical therapy in the patients (use of 3 or more topical medications); (4) existence of ocular allergy and (5) bad compliance. Preoperative data included demographic characteristics, type of glaucoma, preoperative medication and its duration and IOP. Preoperatively, all patients underwent a standard ophthalmic examination including visual acuity (Snellen chart), slit-lamp biomicroscopy, gonioscopy, IOP measurement with Goldmann applanation tonometry and fundus examination using indirect ophthalmoscopy with Volk Superfield lens 90D. The diagnostic observation also included a visual field test using the Threshold C 24-2 Swedish Interactive Testing Algorithm (SITA) standard program using the Humphrey visual field analyzer II (Carl Zeiss, Germany) and scanning laser ophthalmoscopy – Heidelberg retinal tomography (HRT II, Heidelberg Engineering, GmbH, Dossenheim, Germany, version 2.02) exam at least once a year. The diagnoses of POAG and XFG were based on the preoperative definitions: POAG – an optic neuropathy with typical matching disc and glaucoma visual field changes in the presence of an IOP ≥ 22 mmHg without medication and with the gonioscopy finding of wide and open anterior chamber angle and XFG – the same clinical characteristics with the presence of pseudoexfoliation [17]. All operations were performed by five surgeons. All five surgeons used the same technique, fornix based. Routine postoperative management included antibiotic drops for 1 week and topical steroids, 4 times daily, for several weeks. The IOP measurements were performed at the first postoperative day, after 7 days, then 1, 3, 6 and 12 months after the intervention as well at the last obtainable follow-up visit which was within 3 to 5 years. At the last follow-up visit, IOP information was recorded, medications used and any additional operations during the follow-up.

Definition

Successful control of IOP was defined as achieving IOP ≤ 21 mmHg without medication (complete success), or with a single topical medication (qualified success). Failure was defined by IOP > 21 mmHg, IOP ≤ 21 mmHg achieved with more than a single topical medication and requirements for further glaucoma surgery.

All patients signed the informed consent to use their data for the analysis. This study was approved by the Ethics Committee of the Clinical Center of Serbia.

Statistical analysis

Standard descriptive statistics were used. The Kaplan-Meier curves were constructed to assess a successful control of IOP after surgery during the follow-up period. The unpaired Student's *t*-test was used for comparison of the continuous variables. The χ^2 or Fisher's exact tests were used to evaluate the significance of the differences between the categorized data. The Cox univariate and multivariate analyses were performed to assess the predictors of surgery success. Individual differences were considered to be statistically significant for $p < 0.05$. The SPSS version 21.0 (SPSS Inc, Chicago, Ill) was used for all statistical calculations.

Results

The mean duration of preoperative glaucoma medication for the POAG group was 36 months, range 4–360 months (median, 25th–75th percentile) and 24 months, range 3–120 months (median, 25th–75th percentile) for the XFG group ($p < 0.001$).

The patients' characteristics according to the type of glaucoma and the number of preoperative medications are listed in Table 1. The mean IOP preoperatively was 28.4 ± 6.3 mmHg (range 16–48 mmHg) in the POAG group and 30.4 ± 8.4 mmHg (range 17–60 mmHg) in the XFG group ($p = 0.311$). No significant differences were found in the number of topical ($p = 0.085$) and oral ($p = 0.221$) preoperative medicines between the studied group.

One week postoperatively, the mean IOP was 14.6 ± 3.7 mmHg in the POAG group and 14.9 ± 4.3 mmHg in the XFG group ($p = 0.857$) with a statistically significant IOP reduction from preoperatively IOP in both groups: POAG ($p = 0.001$) and XFG ($p = 0.001$).

The mean follow-up period in the POAG group was 50 ± 5 months and in the XFG group was 49 ± 6 months ($p = 0.915$). There was no difference at last IOP according to the type of glaucoma: 16.9 ± 5.2 mmHg (POAG) and 18.7 ± 5.9 mmHg (XFG) ($p = 0.681$). However, there was a significant IOP reduction level at last visit in both groups: 11.5 ± 8.9 mmHg in the POAG group ($p < 0.001$) and 11.7 ± 9.3 mmHg ($p < 0.001$) in the XFG group. There were no statistically significant differences between the POAG and XFG group in the number of postoperative topical ($p = 0.604$) and oral ($p = 0.081$) medicines. The patients' characteristics at last visit are listed in Table 2.

Table 1

Patients' characteristics according to type of glaucoma and the number of preoperative medications

Variables	POAG	XFG	*p*
Number of patients, n	174	158	
Age (years), mean ± SD	64.0 ± 8.6	70.7 ± 8.9	0.001
Male/female, n (%)	85/89 (49/51)	82/76 (52/48)	0.715
Number of eyes, n	188	164	
Duration of preoperative glaucoma medication (months), median; 25th–75th percentile	36; 24–84	24; 12–48	0.023
Preoperative IOP (mmHg), mean ± SD	28.4 ± 6.3	30.4 ± 8.4	0.311
Number of preoperative medicines, n (%)			
topical			
0	6 (3.2)	0 (0)	0.085
1	16 (8.5)	5 (3.1)	
2	48 (25.5)	37 (22.6)	
3	113 (60.1)	116 (70.7)	
4	5 (2.7)	6 (3.6)	
oral	40 (21.2)	46 (28)	0.221

IOP – intraocular pressure; POAG – primary open-angle glaucoma; XFG – pseudoexfoliative glaucoma; n (%) – number (percentage) of patients; SD – standard deviation.

Table 2

Patients' characteristics at the last examination

Variables	POAG	XFG	*p*
Numbers of patients	174	158	
Number of eyes	188	164	
Follow-up after trabeculectomy (months), mean ± SD	50 ± 5	49 ± 6	0.915
Last IOP (mmHg), mean ± SD	16.9 ± 5.2	18.7 ± 5.9	0.681
Reduction in IOP from preoperative level (mmHg), mean ± SD	11.5 ± 8.9	11.7 ± 9.3	0.652
Number of postoperative medicines, n (%)			
topical			
0	110 (58.5)	85 (51.8)	0.604
1	26 (13.8)	33 (20.1)	
≥ 2	52 (27.7)	46 (28.1)	
oral	4 (2.1)	8 (4.9)	0.081

POAG – primary open-angle glaucoma; XFG – pseudoexfoliative glaucoma; IOP – intraocular pressure; n (%) – number (percentage) of patients; SD – standard deviation.

According to the Kaplan-Meier survival curve, the complete success in the group with POAG at 1, 3 and 5 years were 85%, 75% and 58% and in the group with XFG were 82%, 70% and 56%, respectively ($p > 0.05$) (Figure 1). In addition, the complete and qualified successes were 89%, 78% and 69% in the POAG group and 85%, 75% and 66% in the XFG group, respectively (Figure 2).

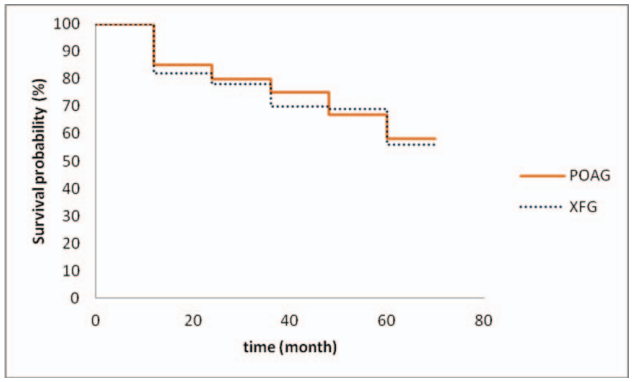

Fig. 1 – Kaplan-Meier curves depicting complete a success in the groups with the primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (XFG).

The Cox univariate analysis which included age, gender, duration of glaucoma, number of preoperative medications, preoperative IOP, glaucoma diagnosis (POAG or XFG) and postoperative complications, revealed that the presence of XFG ($p = 0.018$) and higher preoperative IOP ($p = 0.031$) were associated with the decreased complete success. In addition, the Cox multivariate analysis showed that the presence of XFG was associated with the decreased complete success (HR = 2.612; $p = 0.043$). The characteristics between the patients considering a complete success, a qualified success or a failure at the last examination are listed in Table 3 and Table 4.

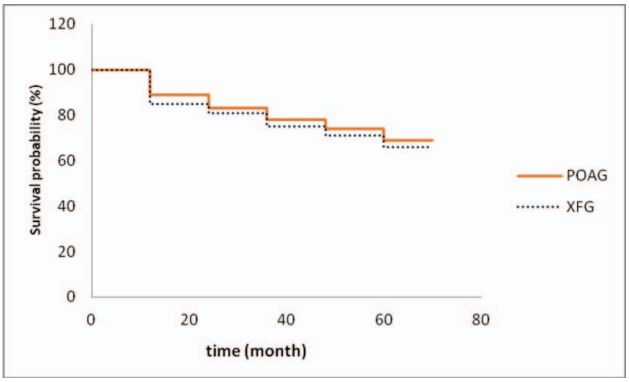

Fig. 2 – Kaplan-Meier curves depicting a complete and qualified success in the groups with the primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (XFG).

Table 3
Characteristics of the patients considered a complete success, a qualified success or a failure at the last examination in the group with primary open-angle glaucoma (POAG)

Variable	Complete	Qualified	Failure
Number of patients (%)	102 (59)	16 (9)	56 (32)
Age (years), mean ± SD	64.7 ± 7.7	65.4 ± 5.6	64.3 ± 6.2
Male/female, n (%)	54/48 (53/47)	6/10 (38/62)	27/29 (48/52)
Number of eyes, n (%)	107 (57)	21 (11)	60 (32)
Number of drops preoperatively, mean ± SD	2.5 ± 1.5	2.8 ± 1.3	2.6 ± 0.5
Duration of preoperative glaucoma medication (months), median	48	24	36
Preoperative IOP (mmHg), mean ± SD	27.5 ± 5.2	26.6 ± 5.5	30.6 ± 5.1
IOP after 7 days (mmHg), mean ± SD	13.6 ± 3.5	15.4 ± 4.5	14.8 ± 3.7
Last IOP (mmHg), mean ± SD	15.1 ± 4.1	18.1 ± 2.1	20.2 ± 2.9
Reduction (mmHg), mean ± SD	12.4 ± 8.5	8.5 ± 5.5	10.4 ± 9.7

IOP – intraocular pressure; SD – standard deviation.

Table 4
Characteristics of the patients considered a complete success, a qualified success or a failure at the last examination in the group with pseudoexfoliation glaucoma (XFG)

Variable	Complete	Qualified	Failure
Number of patients, n (%)	77 (49)	15 (9)	66 (42)
Age (years), mean ± SD	72.7 ± 6.8	70.9 ± 9.1	70.3 ± 7.2
Male/female, n (%)	43/34 (56/44)	9/6 (60/40)	30/36 (45/55)
Number of eyes, n (%)	78 (47)	19 (12)	67 (41)
Number of drops preoperatively, (mean ± SD)	2.6 ± 1.2	2.8 ± 0.8	2.9 ± 1.3
Duration of preoperative glaucoma medication (months), median	36	24	32
Preoperative IOP (mmHg), mean ± SD	28.6 ± 5.6	30.9 ± 5.9	32.1 ± 5.2
IOP after 7 days (mmHg), mean ± SD	14.7 ± 4.4	14.9 ± 3.8	15.1 ± 3.4
Last IOP (mmHg), mean ± SD	16.2 ± 3.1	18.3 ± 2.2	21.2 ± 2.4
Reduction (mmHg), mean ± SD	12.4 ± 8.5	12.6 ± 6.1	10.9 ± 7.3

IOP – intraocular pressure; SD – standard deviation.

Postostoperative complications

In our study, the early postoperative complications were common; in the whole study group, the early postoperative complications included hyphema in 16%, hypotony in 21%, a shallow or flat anterior chamber (AC) in 15% and choroidal detachment in 15%, with some of these complications occurring simultaneously. The other postoperative complications were leakage of filtering bleb in 5%, fibrinous reaction in AC in 2%, early postoperative IOP elevation > 30 mmHg where the only significant predictor of failure was increased IOP at the end of the first postoperative month ($p = 0.025$).

Discussion

The main purpose of this study was to determine success rate in the POAG and XFG patients for a long-term IOP control after primary trabeculectomy and the need for further antiglaucomatous treatment, either medical or surgical. The complete success in the POAG group at 1, 3 and 5 years were 85%, 75% and 58% and in the XFG group were 82%, 70% and 56%, respectively.

Glaucoma represents a significant public health concern and if it is left untreated, it can result in progressive optic nerve damage leading to blindness, often without other symptoms [18]. Controlling IOP has been the primary focus of glaucoma treatment. Indications for glaucoma surgical treatment includes visual field deterioration, or progressive optic neuropathy, despite maximum medical therapy, laser therapy, or both [19].

In order to define the success of surgery, it would be ideal to include an assessment of the visual field and the optic disc stability. A major problem is the time required to collect these data and the test frequency to detect progression. To overcome this problem, the IOP control is often used as a surrogate measure of disease stability and the most frequently used measure of success in the studies of glaucoma surgery [20].

In the early days of trabeculectomy, several authors focused on the IOP-reducing effect of the surgery [13, 14, 21]. Watson and Grierson [13] defined the success criteria IOP ≤ 21 mmHg, and found 86% of success with surgery alone and 98% with the use of additional medical therapy and/or surgery in the 10-year study period (424 eyes). Nouri-Mahdavi et al. [14] identified the success rates of 48% and 40% at 3 and 5 years, respectively, with the defined success criteria IOP ≤ 20 mmHg. Cvetkovic et al. [21] analyzed the effect of 100 trepanotrabeculectomy in the primary (79 cases), congenital (16 cases) and the secondary (5 cases) types of glaucoma and the outcome was as follows: in 87% cases, the intraocular pressure was normalized, in 8% the intraocular pressure was normalized with additional medical therapy after surgery, in 3% the pressure could not be controlled even with the additional medicines and in 2% they got hypotony.

No standard definition exists for the success of glaucoma surgery regarding the IOP, because no single target pressure can be achieved as a safe limit for the disease control [22]. We used a limit of 21 mmHg, because it makes this study comparable with most of other studies dealing with the same problem [7, 10, 15, 16].

Khalili et al. [15] defined the same values (IOP < 21 mmHg), although the results were not promising: at 1, 3 and 5 years, the surgery success were 61%, 50% and 38% respectively for all type of glaucoma. The one of possible explanations for such results were the study criteria. They included all types of glaucoma, i.e., neovascular glaucoma which is more refractive on surgery.

Using the same criteria (IOP ≤ 21 mmHg), Ehrnrooth et al. [16] had a less successful outcome; complete success after 1, 2, 3, 4 years were 63%, 54%, 45% and 40%, respectively. However, in this series, the success was not calculated separately according to the type of glaucoma (POAG and XFG).

In recently published study by Molteno et al. [23], the complete success at 1, 2, 5, 10, 15 and 20 years were 95%, 93%, 89%, 82%, 74% and 68%, respectively. Some of the studies with the success rates of IOP after trabeculectomy at the end of the study period that were not mentioned in the text are listed in Table 5.

Follow-up after trabeculectomy

In our study, the mean follow-up was 4 years (range 3–5 years). In a majority of published papers the surveillance time was similar as the one in our study [12, 14]. The advantage of long-term follow-up facilitates better insight of re-introducing the therapy during the monitoring period.

In some papers, the follow-up was 7 to 10 years [29] , and few authors published the follow-up periods exceeding 10 years [8, 9, 11, 30, 31]. In a study by Popovic and Sjöstrand [30], a success rate of IOP control (≤ 21 mmHg) with, or without additional treatment was analyzed in 75 patients. The follow-up after trabeculectomy lasted 6–12 years. The proportion of patients without medications decreased linearly with time from approximately 90% to 60% between 1 and 10 years postoperatively.

Table 5

Success rates of intraocular pressure control after trabeculectomy in the studies at the end of the study period

Study	Number of patients (eyes)	Follow up (years)	Complete (qualified), n (%)
Edmunds et al. [24]	1,240 (1,240)	1	84 (92)
Law et al. [25]	67 (75)	3	47 (68)
Casson et al. [26]	20 (21)	5	67 (90)
Diestelhorst et al. [27]	547 (700)	10	35 (44)
Molteno et al. [28]	193 (289)	15	n/a (85)
Bevin et al. [9]	607 (841)	20	n/a (79)

n/a – not available.

Table 6
Success rates of intraocular pressure (IOP) control after trabeculectomy in the eyes with primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (XFG)

Study	N* POAG/XFG	Follow up (years)	Complete success, (%) POAG/XFG
Popovic and Sjostrand [30]	23/21	1	78/81
Edmunds et al. [24]	1,105/64	1	66/70
Serguhn and Spiegel [33]	17/21	2	71/86
Mietz et al. [34]	209/117	5	53/51
Tornqvist and Drolsum [29]	56/107	5	43/64
Mills et al. [12]	220/14	7	73/64

*** N – number of eyes.**

Watson et al. [31] followed up their patients for 22 years, or until the patients died; the probability of successful IOP control (< 20 mmHg) with, or without medication after trabeculectomy, was 96%, 86% and 79% at 1, 10 and 20 years, respectively. Landers et al. [8] found at the end of the 20-year period that approximately 60% patients still had a successful control with no additional therapy and approximately 90% with additional medications.

Using mitomycin-C

MMC is used frequently in the patients undergoing glaucoma filtration surgery to prevent the postoperative conjunctival scarring in order to reduce a possibility of failure of the surgery and to reach a low target pressure [17].

Although it is considered mandatory to use MMC in trabeculectomy, the legal obstacles for MMC off-label use in our hospital group (there is an explicit ban on use off-label MMC for trabeculectomy by our hospital group Ethics Committee which comprises of non-ophthalmologists only). This provided a unique opportunity to analyse a fairly large group of patients for a sufficiently long period enabling us to consider if MMC use is necessary or clearly advantageous in all subsets of patients (primarily regarding age).

Types of glaucoma

Several studies showed a success rate after trabeculectomy only in the patients with POAG; ranging from 65% to 83% without medication and 77% to 98% with the use of additional medical therapy or surgery [13]. On the other hand, similar to our criteria, some studies comparing the results of trabeculectomy in the POAG and XFG patients are listed in Table 6.

In our study, the only factor found to be related with a poor long-term control of IOP in the multivariate analysis was the presence of XFG. Previous studies suggested that an inadequate long-term control of IOP may relate to other various factors, such as the type and/or length of preoperative medication and preoperative laser interventions. In the present study, the patients in the XFG group were significantly older and had a significantly shorter duration of preoperative period than the patients in the POAG group, but neither of these factors proved to be a significant predictor of failure in our series.

In our study, early postoperative complications after trabeculectomy were common, but the only complication associated with a failure was the elevated IOP at the end of the first postoperative month resulting from the insufficient filtration, since we know the subconjunctival scarring is more frequent without the use of antimetabolite. Early postoperative hypotony (21%) was less frequent than the range of 24%–39% reported in various studies about trabeculectomy with MMC using differing definitions [9]. In this series, early postoperative hypotony was not associated with an increased rate of failure. In our study, the leakage of filtering bleb occured in 5% of the whole studied group, while in the literature, the bleb leak was not consistently reported where it was mentioned that the rate varied from nil to 20.4% [32]. It is possible that the obligatory use of antimetabolite is responsible for more frequent complications such as filtering bleb, and therefore the awareness of antimetabolite complications may initiate more vigilance in this regard. Cataract development and its progression is a well-documented complication of trabeculectomy, considered as a late complication of trabeculectomy as also a long-term use of topical steroids. The results of this study, like other long-term studies [16, 26, 30], confirmed that trabeculectomy is a safe operation with a low rate of postoperative complications that offers a good long-term control of IOP in most cases with the rate of failure decreasing with increasing length of following-up. Our study has some limitations. The first one is that the success of trabeculectomy was measured only by the single criteria of IOP. The second limitation is that changes in the visual filed and structural characteristics of the subjects' pre and postoperatively was not mentioned. The analysis of patients regarding the stage of their disease separately was not made, too. Also, it should be noted that the operations were performed by five surgeons; therefore, the difference in their individual success should have been considered, too. An important limitation of the present study is that glaucomatous damage stage, criteria for patient selection and definition success after the surgery varied among the studies and influenced significantly the complete findings. There is difference in the qualified success; in our sudy qualified success of IOP was defined as achieving the IOP $\leq$ 21 mmHg with a single topical medication and in some papers as the IOP $\leq$ 21 mm with one or more topical medications. In addition, it should be taken into account that all trabeculectomies were done without the use of MMC from the reasons already explained above, while

use of MMC is mandatory. Finally, it is important to highlight that in the period between January 2007 and December 2009 more patients had trabeculectomy at the Clinic for Eye Diseases in Belgrade than the number included in our study due to the fact that only those who had follow-up period ≥ 3 year were included.

As it was described above, the primary purpose of the surgery was to stop progress of glaucoma, preferably without an additional therapy. For the elderly patients with the poor general condition the satisfactory IOP control without medication could be the main goal of glaucoma surgery, due to the side-effects of medication, or a weak compliance with the use of topical medicines for glaucoma.

Conclusion

Overall IOP control in the both our patient groups (POAG and XFG) was similar to control reported previously. In our study, the complete success in the group with POAG was achieved in 75% and 58% of the patients in the period 3 and 5 years after surgery, respectively and in the group with XFG complete success was achieved in 70% and 56% of the patients, respectively without a statistically significant difference between the POAG and XFG group. In our study, similar to what was reported previously on the same issue, the IOP reducing effect of trabeculectomy was decreasing gradually.

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Factors influencing extent of nausea in the patients on oral iron therapy

Faktori koji utiču na stepen mučnine kod pacijenata na terapiji oralnim preparatima gvožđa

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Abstract

Background/Aim. Nausea after oral administration of iron is frequent phenomenon (11% of patients) and it is believed to be consequence of accumulation of free radicals in mucosa of gastrointestinal tract. The aim of our study was to measure the extent of nausea in outpatients taking oral supplementation with iron, and to investigate possible factors that may have an influence on it. **Methods.** The study was of the cross-sectional type, and conducted on a sample of outpatients on oral iron supplementation. The sample was consecutive, including all patients coming to a community pharmacy for oral iron supply during the study period. Frequency and severity of nausea were measured by the 5-item Drug-Induced Nausea Scale (DINS). **Results.** The mean score of the DINS from the sample of 128 patients was 8.56 ± 5.07 (range from 5 to 25). Each additional cup of coffee per week increased the DINS score for 0.143 points, the history of gastrointestinal disease had protective effect and decreased the DINS score for 5.923 points. **Conclusion.** Frequency and severity of oral iron-induced nausea are not dependent on oral iron burden, but rather on coffee intake and previous experience of patients with symptoms of gastrointestinal diseases. Modification of diet and education about types and severity of symptoms of gastrointestinal diseases could be useful preventive measures to avoid or at least mitigate oral iron-induced nausea and/or vomiting.

Key words:
iron; administration, oral; nausea; risk factors.

Apstrakt

Uvod/Cilj. Mučnina posle oralne primene preparata gvožđa je česta pojava (11% bolesnika). Veruje se da je posledica akumulacije slobodnih radikala u sluzokoži gastrointestinalnog trakta. Cilj istraživanja bio je da se ustanovi stepen mučnine kod ambulantnih bolesnika koji su bili na terapiji oralnim preparatima gvožđa i da se istraže mogući faktori koji mogu uticati na njega. **Metode.** Studija preseka sprovedena je na uzorku ambulantnih bolesnika koji su bili na terapiji oralnim preparatima gvožđa. Uzorak je bio slučajan, uključujući sve bolesnike koji su dolazili u apotekarsku ustanovu radi nabavke oralnih preparata gvožđa tokom perioda istraživanja. Učestalost i stepen izraženosti mučnine su mereni pomoću skale (5 parametara) za mučninu izazvanu lekovima (DINS). **Rezultati.** Ustanovljena je srednja vrednost i standardna devijacija (8,56 ± 5,07) u rasponu od 5 do 25 prema DINS. Svaka dodatna šoljica kafe nedeljeno je povećavala DINS rezultat za 0,143 poena, dok je istorija gastrointestinalne (GIT) bolesti imala zaštitni efekat i ispoljila se kroz smanjenje DINS za 5,923 poena. **Zaključak.** Učestalost i stepen izraženosti mučnine izazvane oralnom primenom preparata gvožđa ne zavise od primenjene doze, već od unošenja kafe i prethodnog iskustva bolesnika sa simptomima GIT. Modifikacija ishrane i edukacije o vrstama i ozbiljnosti simptoma gastrointestinalnih oboljenja mogla bi biti korisna preventivna mjera za izbjegavanje ili barem ublažavanje mučnine i/ili povraćanja izazvanih oralnom primenom preparata gvožđa.

Ključne reči:
gvožđe; oralna primena; mučnina; faktori rizika.

Introduction

Nausea after oral administration of iron salts happens in 11% of patients [1], and it is believed to be a consequence of accumulation of free radicals in mucosa of gastrointestinal tract [2]. Almost 50% of patients who take iron salt orally become eventually non-adherent to the treatment, primarily due to gastrointestinal side effects, which makes nausea caused by oral iron salts to be significant public health problem, too [1]. Especially ferrous sulfate causes nausea, about 2.32 times more often than other drugs or placebo [3]. Type of oral iron salt may affect rate and severity of nausea

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as was reported that ferrous salts (Fe^{2+}) were better tolerated, especially ferrous gluconate in liquid form [3]. However, a recent systematic review of efficacy and safety of oral iron preparations did not confirm existence of differences in rate of nausea and vomiting among different iron salts [4]. Other factors that may influence the rate and severity of nausea after oral iron therapy has not been investigated up to date.

The aim of our study was to measure the extent of nausea in outpatients taking oral supplementation with iron and to investigate possible factors that may have an influence on it.

Methods

Our study was of the cross-sectional type and was conducted during 2016 on a sample of outpatients on oral iron supplementation in the town Osečina, Serbia. The sample was consecutive, including all patients coming to a community pharmacy for oral iron supply from January,1st to December, 31st, 2016. The inclusion criteria were: age over 18 and below 75 years, diagnosis of iron deficiency anemia, oral supplementation of iron lasting at least two weeks prior visit to the community pharmacy and literacy. The exclusion criteria were previous gastrectomy, cognitive disorders (score at the Mini-Mental State Examination below 24), mood disorders and mental retardation. An investigator was a pharmacist employed in the same community pharmacy where the study took place. The study was approved by the Ethics Committee of Clinical Center Kragujevac, Serbia. The patients were enrolled only after they had signed the informed consent form.

Existence and extent of oral iron-induced nausea were measured by the 5-item Drug-Induced Nausea Scale (DINS), which we had constructed, with the following questions rated on the 1–5 Likert's scale [5]: 1) Did you feel nausea during the drug therapy?; 2) Did you feel nausea during the drug therapy aways at the same time during a day?; 3) How often did you feel unable to perform your daily activities due to nausea during the drug therapy?; 4) Did your appetite decrease due to nausea during the drug therapy?; and 5) Did you feel an urge to vomit during the drug therapy? Presence of both independent (type of iron salt in an oral preparation, daily dose of iron, timing of oral iron in relation to a meals, timing of oral iron during a day, smoking, intake of alcohol and intake of coffee) and confounding [sex, age, education, employment status, place of living, pregnancy, knowledge about gastrointestinal adverse effects of oral iron, previous experience with nausea after taking drugs orally, comorbidities (diabetes, asthma, chronic obstructive pulmonary disease, chronic heart failure and hypertension), oral intake of other drugs, concomitant gastrointestinal disease (gastroesophageal reflux disease, peptic ulcer, chronic pancreatitis or inflammatory bowel diseases), chronic renal failure and liver cirrhosis] variables was established by an open-ended questionnaire offered to the patients.

Statistics

The data were primarily processed by the descriptive statistics, calculating frequencies and percentages of different values of categorical variables as well as means and standard deviations of continuous variables. The total score of DINS was calculated as a simple summation of scores on individual questions. Effects of independent and confounding variables on the total DINS score were estimated by the multiple linear regression, through sign and size of coefficients of variables with a significant statistical influence. Optimal regression model was established by backward deletion method. All calculations were performed by the Statistical Program for Social Sciences (SPSS), version 18.

Results

Total of 128 patients with iron deficiency anemia and taking oral supplementation with iron took part in the study. Characteristics of the study sample are shown in the Table1.

The mean score of the DINS was 8.56 ± 5.07 (range from 5 to 25). The optimal multiple regression model (R^2 = 0.114, F = 2.204, p = 0.039) after backward deletion included the following variables: oral intake of other drugs, knowledge of adverse effects of oral iron, experience with nausea after oral intake of drugs, sex, average number of coffee cups weekly, history of gastrointestinal disease and type of iron salt. However, only two variables showed a significant influence on the DINS score: average number of coffee cups weekly (B = 0.143, range 0.022–0.264; p = 0.021) and history of gastrointestinal disease (B = -5.923, range -11.814–0.033; p = 0.049).

Discussion

Many drugs have significant potential to induce nausea and/or vomiting. Main center for vomiting in medulla oblongata is stimulated by certain blood-borne substances, by input from nerve endings in gastrointestinal tract and by projections from the chemioreceptor zone. There are several neurotransmitters which are involved in the functioning of center for vomiting: acetylcholine, histamine, 5-hydroxytriptamine, dopamine, endogenous cannabinoids and substance P [6]. The patients receiving cytostatic drugs experience nausea in 10% (low emetogenic drugs) to 90% (highly emetogenic drugs) of chemotherapy sessions [7], while the patients on opioids feel nausea in 48% of cases when these drugs were used for treatment of cancer pain and in 27% when used for postoperative pain [8]. As already mentioned, nausea due to oral iron supplementation is also frequent phenomenon, occurring in 11% of patients [1].

Our study revealed only two factors with a significant influence on frequency and extent of nausea after oral iron supplementation: coffee intake and history of gastrointestinal disease. While each additional cup of coffee per week increased the DINS score for 0.143 points, the history of gastrointestinal disease had protective effect and decreased the DINS score for 5.923 points.

Table 1

Characteristics of study sample

Variable	Number	Values
Sex, n (%)		
male	16	(12.5)
female	112	(87.5)
Age (years), mean ± SD	47.9 ± 17.1	
Education, n (%)		
elementary or no school	34	(26.4)
high school	66	(51.6)
higher education	28	(22.0)
Employment status, n (%)		
employed	52	(40.6)
unemployed	49	(38.3)
retired	27	(21.1)
Place of living, n (%)		
urban	83	(64.8)
rural	45	(35.2)
Pregnancy, n (%)		
yes	16	(12.5)
no	112	(87.5)
Iron salt, n (%)		
ferrous-fumarate	90	(70.3)
ferric-hydroxyde	16	(12.5)
ferrous-gluconate	19	(14.8)
ferrous pyrophosphate	3	(2.4)
Daily dose of iron salt (mg), mean ± SD	480.9 ± 258.3	
Average duration of iron supplementation (months), mean ± SD	15.1 ± 32.6	
Timing of dose in relation to a meal, n (%)		
before a meal	38	(29.7)
during a meal	7	(5.5)
after a meal	68	(53.1)
I do not care	15	(11.7)
Timing of dose during the day, n (%)		
morning	24	(18.8)
late afternoon or evening	9	(7.0)
morning and evening	86	(67.2)
I do not care	9	(7.0)
Knows about the adverse effects of oral iron, n (%)		
yes	70	(54.7)
no	58	(45.3)
Experience with nausea after oral intake of drugs, n (%)		
yes	16	(12.5)
no	112	(87.5)
Comorbidity, n (%)		
yes	53	(41.4)
no		
Oral intake of other drugs, n (%)	75	(58.6)
no	62	(48.4)
cardiovascular drugs	22	(17.2)
psychotropic drugs	6	(4.7)
antidiabetics	5	(3.9)
several drug groups	33	(25.8)
Smoking, n (%)		
yes	27	(21.1)
no	101	(78.9)
Intake of alcohol, n (%)		
yes	5	(3.9)
no	123	(96.1)
Average number of coffee cups weekly, mean ± SD	12.3 ± 7.5	
History of a gastrointestinal disease, n (%)		
yes	3	(2.3)
no	125	(97.7)
History of gastroscopy, n (%)		
yes	11	(8.6)
no	117	(91.4)
Chronic renal failure, n (%)		
yes	0	(0)
no	128	(128)
Liver cirrhosis, n (%)		
yes	0	(0.0)
no	128	(100.0)

n (%) – number (percentage) of patients; SD – standard deviation.

It seems that coffee somehow augmented nausea as an adverse effect of drugs. It was shown in a group of patients receiving emetogenic chemotherapy that intense aversion to coffee developed after first cycle of therapy, and the patients avoided to take coffee in order to avoid nausea [9]. The patients who were treated by the prostaglandin inhibitors for premenstrual syndrome in a small observational study benefited from avoidance of coffee and they experienced nausea as side effect of the therapy less frequently [10]. The pregnant females who suffer from morning sickness also have strong aversion to coffee [11]. Mechanism of emetogenic action of coffee remains obscure, but probably caffeine increases cholinergic transmission within the vomiting center, as it was recently shown that it blocked acetylcholinesterase [12].

History of gastrointestinal disease means that a patient has experience with nausea and possibly vomiting. It was shown that some psychological treatments like autogenic training, help patients to control nausea due to motion sickness more effectively [13], which could be an explanation why the patients in our study had lower DINS scores if previously exposed to some gastrointestinal disease. Probably, the experience with nausea helped them to be less anxious when they felt it after taking oral iron, and therefore the score was lower on the DINS.

Our study did not find an association among the type of iron salt, daily dose or dosing regimen with severity of nausea, which are the same findings as those published in two systematic reviews of the studies on this topic [4, 14]. Although it seems logical that oxidative stress imposed to mucosa of the stomach by iron depends on its dose and relation to meals, probably this is not the only mechanism by which oral iron causes nausea. It was shown that iron produced a clearly different sensation from the traditional basic tastes, including both olfactory and oral sensations [15], so the taste of metal instead of gastric irritation may be the main factor in pathogenesis of oral iron-induced nausea. Whether intermittent (once weekly) iron supplementation could decrease problems with nausea in comparison to daily iron intake remains unclear and further studies are necessary to answer this question [14, 16].

Limitations of the study

Our results should be taken with caution, since almost two-thirds of patients did not intake iron before the meal, what is recommended dosing regimen. Non-adherence to the prescribed regimen could have influenced indirectly emetogenic action of oral iron and confounded the effects of other factors, including coffee and experience with gastrointestinal diseases.

Conclusion

Frequency and severity of oral iron-induced nausea are not dependent on oral iron burden, but rather on coffee intake and previous experience of patients with symptoms of gastrointestinal diseases. Modification of diet (avoidance of coffee during oral iron supplementation) and educating the patient about types and severity of symptoms of gastrointestinal diseases could be useful preventive measures to avoid or at least mitigate oral iron-induced nausea and/or vomiting.

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How to identify risk for cerebral hyperperfusion syndrome after carotid revascularization procedures

Kako identifikovati rizik od nastanka sindroma cerebralne hiperperfuzije nakon procedura karotidne revaskularizacije

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Key words:
endarterectomy, carotid; cerebral revascularization; risk factors; hypertension; vascular headaches; cerebrovascular disorders.

Ključne reči:
endarterektomija a.carotis; mozak, revaskularizacija; faktori rizika; hipertenzija; glavobolja, vaskularna; cerebrovaskularni poremećaji.

Introduction

Atherosclerotic lesions of the extracranial part of carotid arteries are one of the most common causes of stroke [1]. Carotid endarterectomy (CEA) and carotid stenting (CAS) are therapeutic options in the prevention of primary and secondary stroke in the patients with significant stenosis of internal carotid arteries (ICA) [2]. Although both of these procedures are considered to be relatively safe, there are certain neurological and non-neurological complications. The more serious one is the cerebral hyperperfusion syndrome (CHS). Albeit rare, CHS is a potentially devastating event that can even be fatal if intracranial hemorrhage (ICH) occurs [3–7]. In this review, we will summarize available data regarding this phenomenon and focus on its pathophysiology, prevention, diagnostics and management.

Definition of hyperperfusion and cerebral hyperperfusion syndrome

First and foremost, we must differentiate between the concept of hyperperfusion and CHS. In general, hyperperfusion occurs when cerebral blood flow (CBF) in the revascularized territory increases by 100%, or more, with respect to the baseline values [8, 9]. However, not every patient with hyperperfusion develops CHS. The term CHS was used to describe the clinical entity consisting of symptoms triad: ipsilateral migraine-like headache, seizure, and transient focal neurologic deficits in the absence of cerebral ischemia in combination with the high post-procedural blood pressure (BP). This was first described by Sundt et al. [10].

Pathophysiology

Although this phenomenon is well described in the relevant literature, not much is known about the pathophysiological mechanisms which lead to CHS. As mentioned before, not all patients with increased CBF develop CHS. In the series conducted by Ogasawara et al. [11], 16.7%–28.6% of the patients with an increase in CBF 100% developed CHS. Also, in some cases with slightly elevated CBF, CHS can be developed [11, 12]. That leads to the conclusion that other factors play a role in the occurrence of CHS.

All authors agree that two interlinked and synergized mechanisms lead to increased CBF; first, impaired cerebral autoregulation, and second, the increased postprocedural BP [13, 14].

The main autoregulatory mechanism is the cerebrovascular reactivity (CVR), the ability of the arterioles to constrict or dilate in response to the alterations of blood flow, or to other stimuli (i.e., hypocapnia) [13]. In order to compensate the reduced blood flow to the brain in the patients with severe ICA, stenosis arteriolae remain in the state of maximal dilation to maintain the sufficient cerebral blood supply.

The severity of CVR impairment is likely due to several different factors – a degree of ipsilateral and contralateral ICA stenosis, an incomplete circle of Willis and insufficient collateral flow [3, 15, 16]. However, the syndrome was described even in the patients without contralateral lesions, thereby gi-

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ving even more significance to the disturbed autoregulation mechanisms that can develop in the region of ipsilateral stenosis even without severe contralateral ICA lesion. After CEA, the increased nitric oxide levels during clamping of ICA and increased oxygen-derived free radicals produced during the restoration of the perfusion pressure are involved in the endothelium dysfunction and the deterioration of autoregulatory mechanisms [17]. Besides, several studies have demonstrated significant elevations in malondialdehyde, diene conjugates, or lipoperoxides, the products of free radical-induced lipid peroxidation, in jugular vein plasma immediately after declamping of the ICA [18].

Increased BP after CEA is largely attributed to the baroreceptor reflex failure after denervation during the procedure. This is especially expressed after bilateral CEA; the baroreflex breakdown induced hypertension leading to an increase of CBF. In contrast, the autoregulation mechanisms are diminished and thus lead to hyperperfusion in the previously hypoperfused tissue. Both cerebral hyperperfusion associated with cerebral edema and the elevated intracranial pressure may lead to an increase in central and peripheral norepinephrine levels and a subsequent further elevation of the systemic blood pressure.

Increased CBF, which cannot be controlled by the autoregulatory mechanisms, leads to the transudation of fluid into the pericapillary astrocytes and interstitium. This results in vasogenic white matter edema, especially in the vertebrobasilar circulation territory of the posterior parietal and occipital regions. New studies on rodent models are trying to shed more light on the mechanisms of occurrence of CHS [19].

Clinical presentation, risk factors and diagnostics

CHS can develop at any time; immediately after the procedure to up to a month later, but the majority of patients develop symptoms within the first few days (mean 5 days) [7, 20, 21]. Although this most commonly appears after CAS and CEA, CHS was described and after the subclavian artery stenting [20] and after the endovascular reconstruction of carotid artery in a high-flow carotid-jugular fistula [21]. The reported incidence rate of CHS and ICH after CEA is 1.9% and 0.37% and 1.16% and 0.74% after CAS [3, 6, 7, 10–12, 22]. The most common symptoms include: headaches, fluctuation of consciousness, confusion and focal neurologic deficit (Table 1).

Table 1
Clinical symptoms of cerebral hyperperfusion syndrome

Symptoms	Incidence (%)
Deterioration of consciousness, confusion	37.1
Headache	30.6
Epileptic disturbances, focal seizures	25.8
Motor disturbances (hemiparesis, hemiplegia)	17.7
Abnormal speech, aphasia	6.4
Nausea	4.8
Intracranial hemorrhage	4.8
Psychotic disorders	3.2
Visual disturbances (hemianopsia)	3.2
Ataxia	1.6

Headaches are usually moderate to severe, ipsilateral to the revascularised artery, pounding and migrenous [23]. Focal neurologic deficit is a result of cerebral edema and usually is transient [6, 15, 24, 25]. It includes the cortex derived symptoms – hemiplegia, hemiparesis, hemianopsia, dysphasia, seizures and less commonly ataxia and visual disorders. Seizures can sometimes present even as status epilepticus [26].

By far the most devastating complication of CHS is ICH. It is a very rare complication, as previously stated, but it is often fatal (36%–63%), and up to 80% of survived patients are left with significant morbidity [15, 27, 28]. Since ICH is associated with CHS, the symptoms of increased intracranial pressure can be present (nausea, vomiting, or altered sensorium) [29]. There is a form of hyperacute ICH that occurs within hours after CAS, and it is almost always unpreventable since it occurs without prodromal signs. It could be a result of rupture of perforating arteries in basal ganglia which are exposed with suddenly normalized perfusion pressure after CAS [30].

The occurrence of CHS is multifactorial, while the cerebral perfusion and autoregulation are individualized in each patient. CBF changes in each patient are variable, and there is no proof that a degree of stenosis is directly linked with the CBF variations [31]. This could be explained by the presence of collateral circulation and the degree of cerebral autoregulation impairment in each patient. Various studies indicated a potential role for risk factors, definitive prediction of subgroups of patients with an increased risk of developing CHS after CEA, or CAS, is not feasible. This point expresses not the ambiguity of the risk factors, but the complexity and the multifactorial contribution in the pathogenesis of the syndrome. The risk factors that were described to be significantly involved in developing CHS are shown in Table 2.

Table 2
Risk factors for developing cerebral hyperperfusion

Risk factors
Preoperative
Long standing hypertension with cerebral microangiopathy
Diabetes mellitus
Older age
Recent contralateral carotid endarterectomy
High-grade ipsilateral stenosis
Contralateral occlusion/high grade stenosis
Incomplete circle of Willis
Attenuated cerebrovascular reactivity after acetazolamide challenge
Perioperative
Intraoperative distal carotid pressure of < 40 mmHg
High doses of volatile halogenated hydrocarbon anesthetics
Periprocedural cerebral infarction
Intraoperative ischemia
Refractory postoperative cerebral hyperperfusion
Postoperative
Postoperative hypertension
Administration of anticoagulants or antiplatelet agents

Some risk factors are identified as more significant than the others in the increase the risk of CHS: low pulsatility index, severe ipsilateral or contralateral carotid disease, bilateral carotid artery stenosis and incomplete circle of Willis [15, 32, 33].

Additionally, one study suggested that interval between two procedures should be no less than 3 months [3]. That study reported 6.6% of patients developing CHS after bilateral CEA within less than 3 months. The authors suggested that inconsistencies in baroreceptor function may be a causative factor for CHS.

The use of anticoagulants and antiplatelet agents is routine after CEA and CAS. In the absence of sufficient data, it remains uncertain whether the use of post-procedure anticoagulation therapy may be associated with an increased risk of developing CHS and ICH [27, 28].

There were several techniques suggested to identify the patients at risk for developing CHS. The most widely available method is transcranial color Doppler (TCD). It is used to determine CBF changes via monitoring cerebral blood flow velocities (CBFV) changes in intracranial vessels. TCD is used to assess cerebrovascular reactivity using vasodilator agents such as acetazolamide, CO_2 inhalation, or the breath holding test [34–36]. The blood flow is severely restricted when there is a critical ICA stenosis present and after being removed, the blood flow increases dramatically. In the patients with badly impaired cerebral autoregulation, this dramatically increases the mean flow velocity in the middle cerebral artery (MCA) [34–36]. The preoperative drop in CBFV is indicative of hypoperfusion and can lead to postoperative hyperperfusion and thus to the CHS.

However, TCD has several limitations. The first one is an insufficient cranial window and the second one is the experience of operater [24, 37]. Despite these drawbacks, the TCD findings should always be evaluated carefully. Results in the TCD studies demonstrate that the blood flow redistribution through the anterior communicating pathway and the ophthalmic artery is achieved, in case of contralateral ICA stenosis, and in the patients with contralateral ICA occlusion through the posterior communicating pathway. Asher et al. [4] reported a significant increase in the mean internal carotid artery volume flow (MICAVF) in all patients with CHS during the symptomatic period. After the symptoms receded, the flow volume returned to normal.

Standard computed tomography (CT) has limited value preoperatively and can be completely normal postoperatively. It can still be useful as a quick tool to remove a suspicion of ICH. Also, the brain edema that can be seen early can be indicative of CHS (Figure 1). A recent study showed the pretreatment CT perfusion imaging (CTP) with acetazolamide challenge could identify the patients at risk for CHS after CAS. CTP maps were assessed for the absolute and relative cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT). Although the CTP parameter that the most accurately identified the patients at risk for HPS was the absolute value of post-acetazolamide MTT, resting MTT was sufficiently accurate [37–47]. Single photon emission CT (SPECT) can detect alterations in the preoperative cerebral perfusion (after administering acetazolamide) [47]. It is also very useful in differentiating between cerebral ischemia and hyperperfusion, and identifying the patients at risk of CEA. However, some studies did not find any correlation between preoperative asymmetry in brain perfusion in rest and CHS [39–42]. Ogasawara et al. [43] suggested that hyperperfusion lasting at least up to three postoperative days on SPECT predisposes to the CHS development.

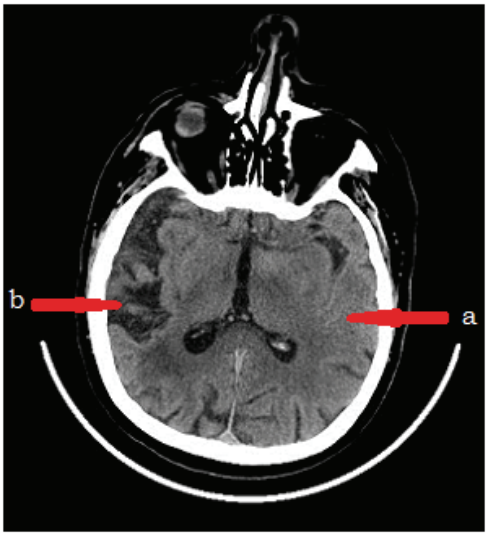

Fig. 1 – Brain computed tomography showing: a) brain swelling as a result of cerebral hyperperfusion syndrome; b) older date ischemic lesion after a stroke.

The magnetic resonance imaging (MRI) techniques also proved useful in diagnosis of CHS, and especially power-weighted (PW) MRI that can reveal intrahemispheric differences in CBF in the patients after CEA [11]. However, as it is not quantitative method, PWI MRI can only be used in absence of contralateral steno-occlusive ICA disease. The conventional MRI findings in the patients with CHS include white matter edema, focal infarction and a local, or a massive hemorrhage. These abnormalities, however, are not pathognomonic for CHS.

Alternative methods such as intraoperative electroencephalography (EEG) and ocular pneumoplethysmography were proposed for diagnosing CHS. However, these methods are yet to prove their worth [48, 49].

Managment of hyperperfusion syndrome

The most important stage in the prevention of CHS is the aggressive BP management postoperatively. This needs to be performed in order to prevent the most dangerous complication of CHS, the ICH. Further reduction of BP even in the normotensive patients should be considered if hyperperfusion is detected, as they can develop hypertension later. Drugs like labetalol and clonidine should be used. Vasodilating drugs with hydralazine, nitrate and Ca^+ channel blockers should be avoided since they can add to already existing brain swelling [23, 42–44]. Beyond this criteria, there is no evidence favoring any other specific drug. Also, beta blockers should be limited [43, 44].

The cerebral edema treatment includes adequate sedation, hyperventilation, manitol administration and hypertonic saline solution [10, 41, 45]. Corticosteroids were tried, but their effectiveness remains uncertain [10, 41, 45].

Oxygen-derived free radicals produced during ischemia were implicated in the ischemia-reperfusion injury. In cerebral tissue, these radicals could lead to the endothelial dysfunction and a break in the blood brain barrier, leading to the post-ischemic hyperperfusion, edema and hemorrhage. In one small case series with historical controls, edaravone, a free-radical scavenger that inhibits lipid peroxidation and vascular endothelial injury decreased the incidence of hyperperfusion following CEA, mainly in the patients with decreased CVR [50].

Conclusion

CHS is a rare, but potentially deadly complication of brain revascularization procedures. Two key, interlinked and synergistic mechanisms play part in its occurrence – impaired cerebral autoregulation and the elevated BP after procedure. It is important to understand the complexity and multiple factors that contribute to the symptom appearance. Although different studies developed risk factors, the determination of subgroups of patients in danger of developing CHS after CAS or CEA is still not feasible. If not treated promptly and properly, CHS can lead to fatal ICH. The treatment strategies were developed towards regulating the BP, reducing brain swelling, and most importantly, limiting the extreme blood flow rising in the patients at potential risk.

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CASE REPORTS

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Triple IgE-positivity to hornet, wasp and bee venom in the patient with anaphylaxis: diagnostic and therapeutic approach

Trostruka IgE-pozitivnost na venome stršljena, ose i pčele kod pacijenta sa anafilaksom: dijagnostički i terapijski pristup

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Abstract

Introduction. Triple-positivity (TP) or double-positivity (DP) for serum-specific immunoglobulin E (sIgE) antibodies against hornet venom (HV), wasp venom (WV) and/or honeybee venom (BV) causes significant problem in a selection of appropriate venom immunotherapy. However, DP/TP can be caused by cross-reactions resulting either from partial sequence identity of protein allergens in the venoms, or may be related to cross-reacting carbohydrate determinants (CCDs). **Case report.** A 60-year-old man was stung by a wasp and two days later by hornet. In both cases, within 15 minutes he developed hypotension and generalized urticaria and he was successfully treated with epinephrine, corticosteroids and fluids. After eight weeks, the examination revealed the negative skin prick test for all three venoms, but the sIgE-determination (ELISA, Biopharm) showed triple sensitization to native BV (0.55 IU/mL), WV (3.35 IU/mL) and HV (0.37 IU/mL). He was receiving the venom immunotherapy with venom mixtures for one year. In order to distinguish true multiple sensitization from cross-reactivity, the molecular-allergy testing by ImmunCAP with the CCD-free recombinant major allergens was performed. A high sensitization to Antigen 5-rVes v5 of WV (31.4 kU/L) was demonstrated while sIgE to phospholipase A2-rApi m1 of BV (0.15 kU/L) was negative; sIgE to CCD-MUXF3-bromelain (0.75 kU/L) explained the sIgE-positivity for native BV. After these findings, a venom immunotherapy only with WV was initiated. **Conclusion.** In our patient, triple-IgE-positivity to native venoms detected by the ELISA was caused by cross-reactivity to CCDs. We recommend the molecular-allergy testing with the nonglycosylated recombinant allergens before starting the venom immunotherapy in patients with multiple-sIgE-positivity to native Hymenoptera venoms.

Key words:
anaphylaxis; bites and stings; cross reactions; desensitization, immunologic; hymenoptera; venoms.

Apstrakt

Uvod. Trostruka pozitivnost (TP) ili dvostruka pozitivnost (DP) serum-specifičnih imunoglobulin E (sIgE) antitela na venom stršljena (SV), venom ose (OV) i/ili venom pčele (PV) izaziva značajan problem u izboru odgovarajućeg venoma za venom imunoterapiju. Međutim, TP/DP može biti prouzrokovana unakrsnim reakcijma koje potiču od delimične identičnosti sekvenci proteinskih alergena ili venomi mogu biti povezani sa unakrsnom reaktivnošću na ugljenhidratne determinante (UHD). **Prikaz bolesnika.** Muškarca starog 60 godina ubola je osa i dva dana kasnije stršljen. U oba slučaja je tokom 15 minuta razvio hipotenziju i generalizovanu urtikariju i bio je uspešno lečen adrenalinom, kortikosteroidima i tečnošću. Posle osam nedelja, pregledom je utvrđeno da je kožni prik test bio negativan na sva tri venoma, ali sIgE određivanje (ELISA, R-Biopharm) je pokazalo trostruku senzibilizaciju na prirodni PV (0.55 IU/mL), OV (3.35 IU/mL) and SV (0.37 IU/mL). On je dobijao venom imunoterapiju sa mešavinom venoma. Da bi se razlikovala prava višestruka preosetljivost od unakrsne reaktivnosti sprovedeno je molekularno-alergološko testiranje sa ImmunoCAP sa alergenima oslobođenim od UHD. Utvrđena je visoka senzibilizacija na Antigen 5-rVes v5 OV (31.4 kU/L), dok je sIgE na phospholipase A2-rApi m1 PV (0.15 kU/L) bio negativan; sIgE na UHD MUXF3-bromelain (0.75 kU/L) je objasnio sIgE-pozitivnost na PV. Nakon ovih nalaza započeta je venom imunoterapijom samo sa OV. **Zaključak.** Trostruka-IgE-pozitivnost na prirodne venome detektovana ELISA testom kod našeg pacijenta je bila prouzrokovana unakrsnom reaktivnošću na UHD. Preporučujemo molekularno-alergološko testiranje sa

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neglikoliziranim rekombinantnim alergenima, pre započinjanja venom imunoterapije kod pacijenata sa višestrukom sIgE-pozitivnošću na prirodne Hymenoptera venome.

Ključne reči:
anafilaksija; ujedi i ubodi; unakrsne reakcije; desenzibilizacija, imunološka; hymenoptera; otrovi.

Introduction

Hymenoptera venom allergy (HVA) is responsible for more than 10% of all cases of anaphylaxis [1]. Identification of the culprit insect from the Hymenoptera venoms in the patients with HVA is often difficult from history because majority of them was unable to identify the insect. The positive testing results for serum-specific immunoglobulin E antibodies (sIgE) to the native Hymenoptera venoms (HVs) components do not always reflect a clinically relevant sensitization. In clinical practice, about 50% of patients with systemic allergic reactions to insect stings show double (DP) or even triple positivity (TP) for HVs, although most of them report the allergic reactions only to one sting. The multiple positive test results can be caused by cross-reactions resulting either from partial sequence identity of protein allergens in the venoms, or may be related to the cross-reacting carbohydrate determinants (CCDs). In the premolecular era, this often led to unnecessary venom immunotherapy (VIT) with more than one venom resulting in the higher costs, increased risk of side effects, unsatisfactory effects of VIT and *de novo* sensitization [1–3]. Nowadays, the molecular allergy testing with the CCD-free recombinant allergens can improve diagnostic precision in the patients with the HVA history, particularly in the patients with multiple sensitization.

Case report

A 60-year-old man presented with the generalized urticaria and hypotension (blood pressure 80/40 mmHg) 15 minutes after having been stung by a wasp in the neck. He was successfully treated at the emergency department with epinephrine, corticosteroids and intravenous fluids and followed up for one day. Two days later, only 5 minutes after a hornet sting, he developed the generalized urticaria, lip swelling and hypotension. Again, he received emergency treatment for anaphylaxis. Then, it took a few days to fully recover after the treatment.

Eight weeks later the skin prick tests (SPT) were performed with the native honey bee venom (BV), wasp venom (WV) and hornet venom (HV) (the Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia), which resulted in the wheal reactions of 1 to 2 mm in diameter for each of the venoms (positive histamine control and negative saline *control* test were included with wheal of 6 mm and 2 mm, respectively). Thus, the SPT were considered negative according to the recommendations [4].

The laboratory testing (ELISA, R-Biopharm, Germany) revealed positive reactions to all three native BV, WV and HV (Table 1). Basal serum tryptase of 4.27 mcg/L was not increased (normal value ≤ 11 mcg/L) (ImmunoCAP, Phadia, Uppsala, Sweden). Thereafter, subcutaneous VIT with the mixture of BV, WV and HV (Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia) was initiated and continued during one year. In order to distinguish the true double/triple sensitization to the HVs from a cross-reactivity, we performed additional testing using the retrieved frozen serum patient sample.

Therefore, we determined sIgE (FEIA, ImmunoCAP, Phadia, Uppsala, Sweden) to recombinant species-specific major allergens (rSSMA) to BV phospholipase A2 (rApi m1), to WV antigen 5 (rVes v5) and to CCDs, MUXF3-CCD-bromelain (Table 1).

The presence of sIgE to antigen 5, but not to phospholipase A_2 ruled out a true double sensitization. Moreover, we found cross-reacting sIgE to CCDs. According to the obtained results we decided to continue the long-term VIT with only WV.

Discussion

HVA carries a high risk of anaphylactic reactions with potentially fatal outcome. It has been shown that 9.2% to 28.7% of the adult population are sensitized to the Hymenoptera venoms and the prevalence of systemic sting reactions ranges between 0.3% and 7.5% [1]. In addition, there is a higher prevalence of more severe systemic reactions to HVs in the patients with the mast cell disorders [5]. Recent studies have demonstrated that the basal serum tryptase levels were elevated in approximately 10% of venom-allergic patients and these increased the levels correlated significantly with severity of Hymenoptera sting and age [6]. The normal basal serum tryptase level in our patient ruled out this possibility.

Table 1

Laboratory diagnostics in the patient stung by the wasp and hornet, two days consecutively

Native allergens	Test values [sIgE (Elisa), IU/mL]	Recombinant major allergens	Test values [sIgE (Immuno CAP-FEIA), kU/L]
Bee venom	0.55	rApi m 1	0.15
Wasp venom	3.35	rVes v 5	31.4
Hornet venom	0.37	Cross reacting carbohydrate determinants (CCDs)	0.75

Normal values: < 0.35, equal for both tests.
FEIA – fluorescence enzyme immunoassay; rApi m 1 rVes v 5 – recombinant phospholipase A_2 and recombinant antigen 5, consecutively.

The most common cause of an insect allergic reaction in Central Europe are stings from honeybee (*Apis mellifera*) and wasp, in the USA called yellow jackets (*Vespula vulgaris*) [3]. The diagnosis of HVA is based on the history of a systemic allergic reaction to insect sting, a positive SPT with the venoms and the presence of sIgE antibodies to the venom. SPT with venoms are the most sensitive for diagnosis of HVA, but can be false negative in less than 2% of patients due to a refractory period of 3–6 weeks, or previous treatment with antihistamines [4, 7]. Still, there are patients with a convincing history of anaphylaxis to Hymenoptera sting, but the negative diagnostic tests to the respected venoms. On the other hand, up to 50% of patients show the positive test results to several venoms [1]. Our patient had a convincing history of anaphylaxis to wasp and hornet stings, the negative SPT results and triple-sIgE-positivity to native BV, WV and HV detected by the ELISA. He was treated for one year by subcutaneous VIT using the mixture of BV, WV and HV. VIT is the most effective treatment for the patients suffering from HVA to avoid life-threatening anaphylaxis. VIT is very effective in inducing tolerance with a protection rate ranging from 75% to 98%. An unnecessary treatment with more than one or even with the wrong venom can lead to *de novo* sensitization and increased risk of side effects [1, 8].

However, a large proportion of patients with allergic reaction to bee, or wasp, or hornet stings have sIgE to the all three venoms. Such multiple positivity causes significant problems in the selection of venoms for immunotherapy. Diagnostic tests sometimes reflect genuine multiple sensitization indicating potential systemic allergic reactions to the next sting by any of mentioned insects. Nevertheless, more often we detect false DP/TP which is clinically irrelevant and can be caused by cross-reactions. The majority of cross-reactivity can be attributed to IgE antibodies that are directed to CCDs which are frequently present in allergens of insects and plants and are present in several allergens of HVs. In insects, the relevant carbohydrate epitope is defined by a α-1, 3-linked fucose residue of the N-glycan. [9, 10]. Another cause of multiple sensitization may be based on the recognition of common protein epitopes of homologous allergens, present in HVs as described for hyaluronidases (Api m 2 and Ves v 2), dipeptidyl peptidases (Api m 5 and Ves v 3) and vitellogenins (Api m 12 and Ves v 6) sharing around 50% sequence identity. The detection of sIgE to CCDs does not allow the exclusion of sensitization to protein epitopes of multiple venoms [10–12].

New molecular-allergy testing based on the detection of IgE antibodies against individual nonglycosylated major Hymenoptera allergens may help to distinguish between the cross-reactivity and genuine multiple sensitization [2, 3, 7]. Many patients with true double sensitization may be identified by means of the rSSMA: rApi m 1 (phospholipase A2) of BV, rVes v 1 (phospholipase A1) and rVesp v 5 (Antigen 5) of WV, that are available for routine *in vivo* diagnostics [13, 14]. We determined sIgE antibodies to major allergens from honey bee (rApi m 1) and the wasp (rVes v 5) venom which are structurally not related. We found sIgE-positivity only for rVes v 5 (Table 1). Elevated sIgE to CCDs explain the finding of increased sIgE levels against the native bee, wasp and hornet venoms (Table 1) detected by the ELISA [2, 3, 7, 15]. A high percentage, even up to 93% of subjects sensitized to the hornet venom have elevated sIgE to rVes v5 [16–18]. Cross-reactivity that occurs between the venoms of different Vespidae (*Vespula vulgaris*, *Vespa* crabo – European hornet) is strong, due to the similarities of venom composition and structure of allergens. Antigen 5 (rVes v 5) is the most potent allergen in the hornet and wasp venom, and the rVes v5 should be helpful for the serological confirmation of hornet and wasp venom sensitization [19]. Highly elevated sIgE to rVes v5 explain systemic reaction provoked by the wasp and hornet sting in our patient. According to our results (Table 1) we changed VIT from the commercial mixture of HVs to a single wasp venom therapy, having in mind that VIT with the wasp venom provides effective protection against both hornet and wasp venom allergy [20, 21].

Conclusion

We recommend molecular-allergy testing to recombinant the major allergens and cross-reactive carbohydrate determinants before the introduction of a long-term allergen-specific immunotherapy for the patients with inconclusive skin tests and/or multiple positive tests for sIgE against native Hymenoptera venoms. The treatment with the primarily sensitizing venom provides more efficient protection and reduces the risks comparing to immunotherapy with venom mixtures.

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CASE REPORT

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The role of an ophthalmologist in the Alström syndrome diagnosis

Uloga oftalmologa u dijagnostici Alstromovog sindroma

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Abstract

Introduction. The Alström syndrome (AS) is an extremely rare autosomal recessive genetic disorder, affecting fewer than 1: 1,000,000 people globally. It is a single gene disorder due to the mutation of *ALMS1* on chromosome 2 (2p13). The AS affects multiple organs and systems. Approximately 800 affected individuals have been identified worldwide so far. Some cases of the AS may go unrecognized for years as many of the clinical features develop over a longer period of time. As the nystagmus and retinitis pigmentosa are the most consistent findings, usually the first visible sign and present at the early infant period, the main aim of this article is to emphasize the importance of the ophthalmologist in establishing an adequate diagnosis of this rare syndrome. **Case report.** This article describes a Serbian patient with the Alström syndrome, whose diagnosis was genetically confirmed using the whole exome sequencing. Our patient was a 7-year-old obese male with symptoms of progressive visual impairment, photophobia and nystagmus diagnosed in early childhood. On admission, the bilateral visual acuity was poor, RE 0.06, LE 0.01, the intraocular pressure within range. The funduscopy showed central retinal pigmentation, thus suggesting cone-rod retinal dystrophy with "bull's eye maculopathy". The initial laboratory work at the time of the consultation revealed the elevated triglycerides levels and hyperinsulinemia, increased transaminases and gamma-glutamyl transpeptidase serum activity, whereas the glucose and glycated hemoglobin (HbA1C) levels were normal. The bilirubin test results were normal. Overall, the clinical manifestations were absent. The patient's cardiac function was normal and the echocardiography did not indicate any abnormalities at the time. His sensorineural hearing was normal as well. A molecular genetic analysis was performed. Two composite heterozygous mutations were discovered within the *ALMS1* gene sequence. In addition to the clinical presentation, the mutation detection confirmed the initial diagnosis of the AS. **Conclusion.** The Alström syndrome should be kept in mind in case of an obese child with photophobia, nystagmus and visual impairment present from early childhood. Fundus examination by an ophthalmologist may significantly help to establish the diagnosis of this rare genetic syndrome.

Key words:
alstrom syndrome; diagnosis; ophthalmologists.

Apstrakt

Uvod. Alstromov syndrome (AS) je veoma retko autosomno recesivno oboljenje koje se javlja sa prevalencijom manjom od 1:1,000,000 ljudi širom sveta. To je oboljenje gena *ALMS1* na hromozomu 2 (2p13) koji utiče na više sistema organa. Do sada, oko 800 ljudi širom sveta ima dijagnostikovan AS. Neki slučajevi AS mogu da budu neprepoznati s obzirom na to da se mnoge kliničke karakteristike razvijaju tokom vremena. Kako su nistagmus i distrofija retine najdosledniji nalazi koji se obično pojavljuju prvi, u ranom detinjstvu, želimo da ukažemo na značaj oftalmologa u postavljanju dijagnoze ovog retkog sindroma. **Prikaz bolesnika.** U ovom radu, opisan je bolesnik sa Alstromovim sindromom čija dijagnoza je potvrđena genetskom analizom sekvenciranja celog egzoma. U pitanju je bio sedmogodišnji dečak, gojazan, sa simptomima progresivnog smanjenja vida, fotofobijom i nistagmusom koji su počeli u ranom detinjstvu. Početna oštrina vida je bila na oba oka skromna, desno oko 0,06, levo oko 0,01, intraokularni pritisak u normalnim granicama. Na fundusu se videla centralna retinalna pigmentacija koja je ukazivala na *cone-rod* retinalnu distrofiju sa "bull's eye maculopathy". Laboratorijski nalazi su po blago povišene nivoe triglicerida. Glukoza i glikozilirani hemoglobin (HbA1C) su bili u normalnim granicama, ali je bila prisutna hiperinsulinemija. Takođe, aktivnosti transaminaza i gamaglutamil transpeptidaze u serumu su bili povišeni. Vrednosti bilirubina su bile u granicama referentnih vrednosti. Kardiološki i ehokardiografski nalazi su bili uredni. Rezultati ispitivanja sluha su bili u granicama referentih vrednosti. Sprovedena je molekularna genetska analiza. Dve složene heterozigotne mutacije nađene su na *ALMS1* genu koje su, uz prisustvo kliničkih manifestacija, potvrdile dijagnozu AS. **Zaključak.** O Alstromovom sindromu treba razmišljati kada imamo gojazno dete sa fotofobijom, nistagmusom i smanjenjem vida koje datira od ranog detinjstva. Pregled fundusa od strane oftal-

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mologa može da bude od velikog značaja i da podstakne sumnju na ovaj redak genetski sindrom.

Ključne reči:
sindrom, almstrom; dijagnoza; oftalmolozi.

Introduction

The Alström syndrome (AS) is an extremely rare autosomal recessive genetic disorder [1] affecting fewer than 1: 1,000,000 people globally [2]. It is a single gene disorder, due to the mutation of *ALMS1* on chromosome 2 (2p13). The AS affects multiple organs and systems. The characteristic features of this syndrome are cone-rod retinal dystrophy causing juvenile blindness, hearing loss, truncal obesity, hyperinsulinemia and insulin resistance, type 2 diabetes mellitus (T2DM), hypertriglyceridemia, dilated cardiomyopathy, and progressive renal, pulmonary, hepatic and neurological dysfunction with mild seizure activity. These combinations of pathologies lead the patients to require multiple subspecialists appointments in order to prevent further complications [3, 4].

Approximately 800 affected individuals have been identified worldwide so far [5]. However, some cases of AS may go unrecognized or misdiagnosed as many of the clinical features develop over time, as the child grows. Also, there are wide clinical variations among the affected individuals [6]. That makes it difficult to determine the true frequency in the general population. The AS diagnosis is usually made on the basis of established clinical features, depending on the age of the patient [7], often without genetic confirmation. However, in most cases the diagnosis is made retrospectively, usually in the first decade of life, after the development of various extraocular features [8].

Since the nystagmus and retinitis pigmentosa are the most consistent findings, usually present first and in early childhood, the main aim of this article is to emphasize the importance of the ophthalmologist in establishing an adequate diagnosis of this rare syndrome. There are only two cases of the AS reported in Serbia [3]. In this study we want to present the third Serbian patient with the AS whose diagnosis was genetically confirmed using the whole exome sequencing.

Case report

Our case was a 7-year-old male with symptoms of progressive visual impairment, photophobia and nystagmus noted from early childhood. His preliminary diagnosis was cone-rod retinal dystrophy. The patient was born as a second child of a two-child family, from a normal pregnancy and delivery. His family consists of non-related parents, both alive and healthy, and an older sister who is in good health.

This patient underwent the complete physical examinations including the ophthalmological, neurological, otorhynolaryngological and pediatric evaluations. The biochemical investigations as well as molecular genetic analysis were carried out. On admission, the bilateral visual acuity was poor, right eye (RE) 0.06, left eye (LE) 0.01, the intraocular pressure within range. In the neurological examination, both pupils were round in shape and midsize (3.5 mm), with a normal response to light. No abnormalities in the eye movements were detected except the horizontal nystagmus. The fundoscopy showed central retinal pigmentation, thus suggesting cone-rod retinal dystrophy with bull's eye maculopathy (Figures 1 and 2).

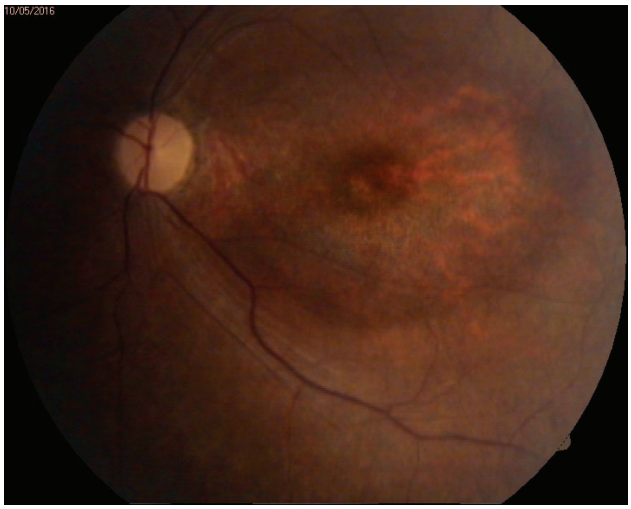

Fig. 1 – Fundus photography of the left eye showing the "bull's eye maculopathy".

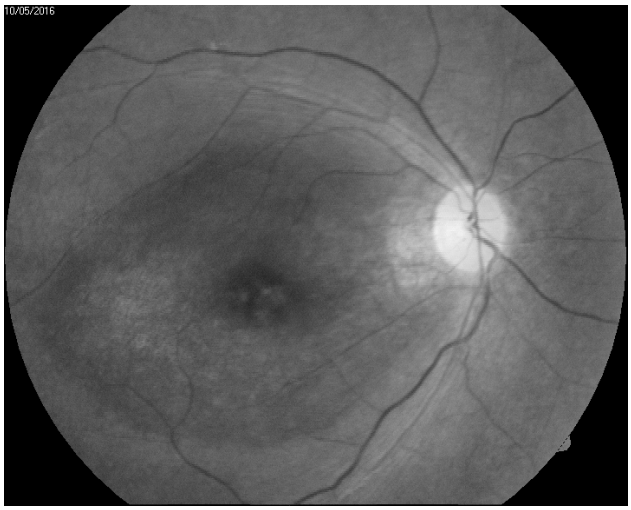

Fig. 2 – Red free photography of the right eye showing the „bull's eye maculopathy".

In the physical examination, there was evidence of central obesity (Figure 3) with height being 119 cm, weight 36.9 kg, body mass index (BMI) 26,1 kg/m^2 (percentile > 98th) and waist circumference 78 cm. His cardiac function was normal as well as his sensorineural hearing. His blood pressure (BP) was 132/86 mmHg (percentile 95th) with a regular pulse of 80 beats per minute.

a)

b)

Fig. 3 – Clinical pictures of a male boy with the Alström syndrome at the age of 7 years, presenting characteristic central obesity: a) characteristic round face; b) characteristic truncal obesity.

The initial laboratory analyses at the time of the consultation revealed a slightly elevated triglycerides level 1.47 mmol/L (normal range 0.34–1.24) with a high density lipoprotein cholesterol (HDL-C) level of 0.99 mmol/L (normal range 1.04–1.55). His glucose and HbA1C levels were normal (4.7 mmol/L and 5%, respectively), while hyperinsulinemia was observed at 31 mU/L (normal < 10 mU/L). His transaminases [aspartate transaminase (AST) and alanine transaminase (ALT)] and gamma-glutamyl transpeptidase (GGT) serum activities were elevated (AST 104U/L, ALT 247U/L, GGT 73U/L). The bilirubin test results were normal. Overall, the clinical manifestations were absent. The abdominal ultrasound showed evidence of steatosis, with normal liver and spleen measurements. Echocardiography did not indicate any pathology at the time. The blood urea nitrogen, creatinine and uric acid were normal. The renal echocardiography did not indicate any abnormalities. His current treatment was a low-fat diet and moderate exercise.

As a result of therapy, after six months, the triglyceride level decreased to 1.1 mmol/L, while HDL-C decreased to 0.93 mmol/L. Despite the diet correction, the child was not physically active. Adding physical activity helped him raise HDL cholesterol to 1.36 mmol/L at the next checkup.

Our patient underwent the molecular genetic analysis (Genetic Diagnostic Laboratory, Strasbourg University Hospitals, France). The coding sequences of exons 16 and 19 of *ALMS1* were polymerase chain reaction (PCR) amplified, purified and products were sequenced according to standard methods. The genetic samples were analyzed in order to characterize retinitis pigmentosa on the molecular plane. As a result, two composite heterozygous mutations were discovered in the gene *ALMS1*: two nucleotide variations in exons 16 and 19 of the gene *ALMS1*: c. [10569_10570del]; [12047del], p. [His3523Glnfs*17]; [Gly4016Alafs*15]). A heterozygous *ALMS1* mutation detected in exon 16 and 19, together with the clinical picture, confirmed the AS diagnosis.

Discussion

The aim of this article is to highlight the AS as one of the rarest genetic disorders in the world. This syndrome represents a ciliopathy that involves multiple organs and shares mutual clinical characteristics of blindness due to retinal dystrophy, early onset of obesity, diabetes and neurosensorial deafness [9,10]. Although the specific role of the *ALMS1* protein has not yet been thoroughly investigated, it plays an important role in the cilia function and intraflagellar transport, allowing the AS to be classified as a ciliopathy [11]. Therefore, it is pertinent to distinguish the AS from other similar diseases accompanied by childhood obesity and retinal dystrophy, such as the Bardet-Biedl and Laurence-Moon syndromes. The cognitive impairment and distal digit abnormalities separate it from a more common ciliopathy, the Bardet-Biedl, while deafness and the absence of spastic paraparesis differentiate the AS from Laurence-Moon [12]. The exact etiology of the disease is unknown, but it is believed to be due to progressive multiorgan fibrosis that leads to organ failure, which is the major cause of morbidity and mortality in the AS [6].

As some clinical features of AS do not become apparent until adolescence, an early diagnosis can be difficult in young children. The AS exhibits a great extent of clinical variability, thus creating difficulties for the general definition [13]. Marshall et al. [14] set the major and minor criteria according to ages, which help the physician set a diagnosis. On the other hand, a diagnosis of the AS could be confirmed at any age with the genetic analysis when two *ALMS1* muta-

tions were identified, or one mutated *ALMS1* allele found in the context of characteristic clinical signs [10, 14]. Therefore, the genetic testing should be carried out in the patients who do not have all of the classic AS characteristics [14] and when the combination of the recommended criteria does not allow a clinical diagnosis [7]. There is no treatment that can cure the AS, prevent, and/or reverse the clinical features. The patients require a thorough initial assessment along with an intensive multidisciplinary approach and follow-ups in order to detect and treat potential complications [7]. Only an early diagnosis of the AS allows us a timely and preplanned management to avoid undesired complications and thus improve longevity as well as the quality of life of the patients [15]. The most common cause of death is hepatic dysfunction and congestive heart failure while life expectancy rarely exceeds 40 years [7]. Our patient was on a controlled diet and moderate physical exercise that usually lead to the reduction of the body mass index and better lipoprotein values [16].

We suspected the AS in our patient according to the criteria from the second group [7]. The genetic results confirmed the reputed diagnosis of the AS, linked to the locus *ALMS1* previously rendered. The AS is autosomal recessive, so the parents of the affected child represent heterozygous carriers who do not show any signs of the disease. We assume that one of these mutations comes from the mother, while the other one probably from the father, but this was not confirmed since the father's sample was unavailable. The mutation of *ALMS1* gene causes cone-rod retinal dystrophy, which is part of the AS spectrum. The fundus examination gains ever-increasing importance because retinal dystrophy represents the earliest and most constant feature of the AS [17]. Still, retinal dystrophy is not isolated and is accompanied with multiple organ disorders mentioned above [6, 7]. Therefore, the detailed multidisciplinary approach is recommended [18] including the biological exams such as liver and kidney biological functions, search for diabetes, insulin resistance, dyslipidemia, cardiological monitoring, search for deafness and overweight care. Prenatal and predictive diagnosis should be conducted if both mutated alleles are found in the parents [14].

Conclusion

In conclusion, the Alström syndrome should be kept in mind while examining an obese child with photophobia, nystagmus and visual impairment present from early childhood. Genetic analysis is quite difficult and expensive, so all patients with diabetes or truncal obesity are not able to apply. The fundus examination by an ophthalmologist can be of a significant help in pointing out the diagnosis of this rare genetic syndrome especially as there is no cure for this condition and detailed multidisciplinary approach is recommended in order to prevent further complications.

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The work of a German oncologist Ferdinand Blumenthal in the Kingdom of Yugoslavia, 1933–1937

Rad nemačkog onkologa Ferdinanda Blumentala u Kraljevini Jugoslaviji od 1933. do 1937.

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Key words:
history of medicine; medical oncology; neoplasms; yugoslavia.

Ključne reči:
istorija medicine; onkologija; neoplazme; jugoslavija.

Introduction

After the adoption of racial laws in Germany, the well-known German oncologist Ferdinand Blumenthal (Figure 1), one of the founders of cancer research in Germany, left the Third Reich and arrived in Belgrade in October 1933. The German oncologist working in Yugoslavia between the wars is interesting for the history of the development of Yugoslav cancer research, which at the time was still in its infancy.

Fig. 1 – Ferdinand Blumenthal.

Blumenthal: one of the founders of cancer research in Germany

Ferdinand Blumenthal was born into a German Jewish family in Berlin on June 5, 1870. He studied medicine in Freiburg, Strasbourg, Zurich and Berlin, and completed his doctorate in 1895 in Freiburg after publishing his research under the title *Über den Einfluss des Alkalis auf den Stoffwechsel der Microben* (The Influence of Alkali on the Metabolism of Microbes). He started as an internist at the *I. Medizinischen Universitätsklinik der Charité* (First Medical Clinic, Charité University Hospital) in Berlin and also conducted research in physiological chemistry and bacteriology. At the beginning of his career, he wrote several studies on putrefaction, cholera, diphtheria and was particularly known for his studies on tetanus, nondiabetic glycosuria and pathological changes in urine. He completed his residency at Friedrich Wilhelm University in Berlin in 1899 and in 1905 he became an associate professor there [1–3].

In the meantime, his research interests were focused on cancer after the first cancer research and treatment center at Charité opened in 1903. This department later became a separate research institute, and for the next three decades Blumenthal played a leading role in its development and also served as an administrator and researcher. His research interests were two-fold: the development and metabolism of cancer cells and the treatment of cancer. He received international recognition in 1910 when he published *Die chemischen Vorgänge bei der Krebskrankheit* (Chemical Processes in Cancer) [4,5].

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He began at the institute as the head of its laboratory from 1903 to 1907, was named a co-director in 1917 and from 1923, he served as the institute's director. In later writings, his colleagues and his biographers placed a particular emphasis on his management skills during World War I, which he used to secure financial stability and continuity of research through donations and private investments. Under his leadership, the institute served as a role model not only in Germany but also in international medical circles, particularly due to being able to provide centralized treatment and care and the inclusion of an outpatient department. He used his authority in the field of cancer treatment to promote the establishment of independent cancer treatment centers, an interdisciplinary approach and multimodality treatment, thus pioneering the approaches and methods later accepted in cancer treatment. He was also an advocate of follow-up treatment for cancer patients [4–6].

In 1919, Ferdinand Blumenthal became the Secretary-General of the *Deutschen Zentralkomitees zur Erforschung und Bekämpfung der Krebskrankheit* (German Central Committee for Cancer Research), a predecessor of the *Deutsche Krebsgesellschaft* (German Cancer Society), and was an editor of the *Zeitschrift für Krebsforschung* (Journal of Cancer Research), which was at that time a renowned journal in the field. His book *Ergebnisse der experimentellen Krebsforschung und Krebstherapie* (Results of Experimental Cancer Research and Cancer Therapy), published in 1934, presented an overview of the state of cancer research. By the time the book was published, however, he was already in exile in the Kingdom of Yugoslavia [2, 4].

Arrival in the Kingdom of Yugoslavia

The global economic crises of 1929-1933 had serious political consequences in Germany, including the rise of political right and the appointment of Adolf Hitler, the leader of the Nazi party, as a German chancellor in January 1933. He then eliminated his partners in the government, banned all political organizations in the country and seized control of the government after the death of German president Hindenburg in 1934 [7]. Shortly thereafter, discrimination began against the political opponents, primarily on the Left, but also followed by discrimination of the Jews. The Nazi's pronounced anti-Semitism turned into a violent pogrom, which later became known as the Holocaust. The violence had been preceded by an aggressive anti-Semitic campaign in the German media, which spurred the emigration of a large proportion of the wealthier Jewish population, including Blumenthal and his family [8]. He left Berlin in the early spring of 1933 and initially settled in Switzerland, still hoping to return to Germany. When he was forced into retirement by the Prussian Ministry for Science, Art, and Education in September 1933, it was obvious that returning to Germany would be impossible, and he went instead to Belgrade in October 1933 [4].

Earlier, in April 1933, a Belgrade medical journal *Medicinski pregled* (The Medical Review) wrote that due to persecution in Germany, a large number of Jews, many of them scientists and more liberally oriented people, would have to leave Germany. Ferdinand Blumenthal, a well-known cancer researcher, oncologist, and director of the University Institute for Cancer Research in Berlin was among them [9]. In May 1933, the Faculty of Medicine at the University of Belgrade invited him to come and work in Belgrade, and soon elected him professor [10]. Blumenthal had visited Belgrade before his October 1933 move, and had met with Radenko Stanković, Minister of Education as well as Vladimir Petković, Rector of the University of Belgrade and Richard Burian, Dean of the Faculty of Medicine. During these meetings, he was given an offer to open a separate Institute for Oncology which would deal with cancer research and treatment. *Politika* and *Vreme*, the most influential Belgrade daily newspapers, reported extensively on the well-known cancer expert's visit. The articles also mentioned that Blumenthal would be named a honorary professor at the Faculty of Medicine and that Blumenthal himself had stated he would not be entering private practice [11]. He also announced his intention to follow in the footsteps of Prof. Đorđe Joannović, the recently deceased founder of the Institute for Pathology in Belgrade [*]. In his first public appearance in Belgrade, Blumenthal praised the Belgrade medical institutions, singling out in particular Đorđe Joannović, whom he considered to be a world-class scientist [12]. After being forced into retirement in Germany, Blumenthal finally arrived in Belgrade in October 1933 [13].

Work in Belgrade

Blumenthal's time in Belgrade (1933–1937) was characterized by the internal political turmoil and the external political context of fascism on the rise in Europe. In the mid-1930s, after the assassination of the King Aleksandar Karađorđević in Marseille in 1934, in a complot evidently supported by fascist Italy, Yugoslavia was leaning towards

[*] Đorđe Joannović was born in Vienna, where he completed primary and secondary school. His family originated from the village of Beodra (modern-day Novo Miloševo) in the Banat. He graduated in 1895 from the Faculty of Medicine in Vienna. He was elected Assistant Professor there in 1904, and was named Associate Professor of Pathology in 1910, and Full Professor in 1919. Although he was the Head of the Pathology Department of the Vienna General Hospital, he left Austria out of a desire to help the newly established Kingdom of Serbs, Croats and Slovenes. He was an active participant in establishing the Institute of Pathology in Belgrade and founded the study of oncology and experimental pathology in Serbia. He was elected as a corresponding member of the Serbian Royal Academy of Sciences, elected to three terms as Dean of the Faculty of Medicine and served as president both of the Serbian Medical Society and the Yugoslav Medical Society. He was also a member of the German and Czech Oncology Committee for the Prevention of Cancer and served in the editorial boards of numerous medical journals, among other activities. He died during an attack by gendarmes on the Faculty of Medicine on January 28, 1932. He clashed with gendarmes who had entered the Faculty while trying to protect his students who were protesting. The exact circumstances of his death are unclear. He was buried in a cemetery in Beodra.

Germany and Italy, particularly during the government of Milan Stojadinović (1935–1939). The Germany's growing political and economic influence led to an increase of anti-Semitism in a part of society and in some of the media, primarily at the right-wing end of the political spectrum, whose rise across Europe was a consequence of the worldwide economic crisis. In public discourse in the Belgrade periodicals from this period, behind verbal disavowals of anti-Semitism, there was an anti-Semitic discourse accompanied by stereotypes of Jewish racial characteristics as well as accusations that the appearance of anti-Semitism within society was a consequence of the Jews' own behavior. In the conservative, nationalist press, anti-Semitism was at first hidden by an insistence on distrust towards foreigners. Over time, it became increasingly more open, it led to an increase in anti-Semitic discourse among the ruling circles, and the results of this were the 1939 anti-Semitic decrees, which came into effect in 1940, concerning the Jewish population's right to education (*numerus clausus*) and limited trade rights, and the first internment camps for left-wing political opponents in Višegrad and Bileća [13]. The decrees came during the time when Blumenthal was no longer in Yugoslavia, but the entire climate that led to their enactment essentially defined the scope and possibilities for Blumenthal's time in Belgrade as well as being one of the reasons for his departure.

When Blumenthal arrived in Belgrade from Switzerland in 1933, the Belgrade newspapers praised him in numerous articles and editorials, stating that his arrival was a significant event for Yugoslav medical science. As had been previously agreed, Blumenthal was expected to establish in Belgrade an institute for cancer research modelled on the institute he had headed in Berlin. He was also expected to centralize and systematize cancer research in the Kingdom of Yugoslavia, stimulate scientific research on the disease and train physicians and students. He was granted a temporary position at the Institute of Physiology within the Faculty of Medicine and was given a permission to bring along one of his assistants from Berlin. At the end of 1933, one of his first tasks was to represent the Kingdom of Yugoslavia at the First International Cancer Congress in Madrid, which became known in 1935 as the International Union against Cancer (and from 2010 as the Union for International Cancer Control) [11, 13–15].

Blumenthal's professional engagement in Belgrade began with a formal introductory lecture at the Institute of Physiology on November 9, 1933. Standing before the professors and students from the Faculty of Medicine, he expressed in Serbian his gratitude to the Yugoslav government, the University of Belgrade, and the Faculty for inviting him to Yugoslavia. He expressed a great pleasure at being in Belgrade, and that it was a great honor to continue the work of Đorđe Joannović, a world-renowned scientist, whose work was "highly recognized" in scientific circles [13, 16].

Throughout 1934 and 1935, Blumenthal led a special cancer infirmary within the *Interna propedevtička klinika* (Propaedeutic Clinic for Internal Medicine) where he received patients twice a week. According to the Dean's annual report, 132 patients were examined between October 1, 1934 and July 1, 1935, of whom 64 were new cases. Patients were given advice on treatment and free medications, or they were sent for surgery and radiation. In a report for the next academic year, Blumenthal examined 207 patients of whom 66 were new cases [13].

During the academic year 1934/1935, he held lectures at the Faculty of Medicine in both German and French. The topics of his lectures were "The Issue of Surgery and Radiation in Cancer Treatment," "Dietary Basics for Cancer Patients," "The Etiology and Biology of Malignant Tumors," and "Diagnostic and Therapeutic Methods in Cancer Treatment and their Critique" [17, 18]. In addition to these, Blumenthal held several noteworthy lectures in Europe between 1934 and 1936 at the medical training courses in San Marino, Lugarno, Milan, Brussels, and Athens, among other places, which usually dealt with the advancement in the diagnosis and treatment of cancer. Blumenthal again represented Yugoslavia as one of the keynote speakers at the Second International Cancer Congress held this time in Brussels in 1936, which attracted around 500 doctors from 42 countries [13, 19].

During his time in Belgrade, Blumenthal published two papers in *The Medical Review*: "Introduction to Experimental Cancer Research" in 1933 and "Basic Principles for Diagnosis of Cancer" (co-authored with Dr. Lazar Stanojević) in 1936 [20, 21]. Stanojević translated Blumenthal's book "Results of Experimental Cancer Research and Cancer Therapy" as *Rezultati eksperimentalnog ispitivanja i lečenja raka* (Figure 2), which was published in 1937 by Geca Kon Publishing House in Belgrade. As stated in the introduction of the Serbian translation, this book was the basis for his lectures at the University of Belgrade [22].

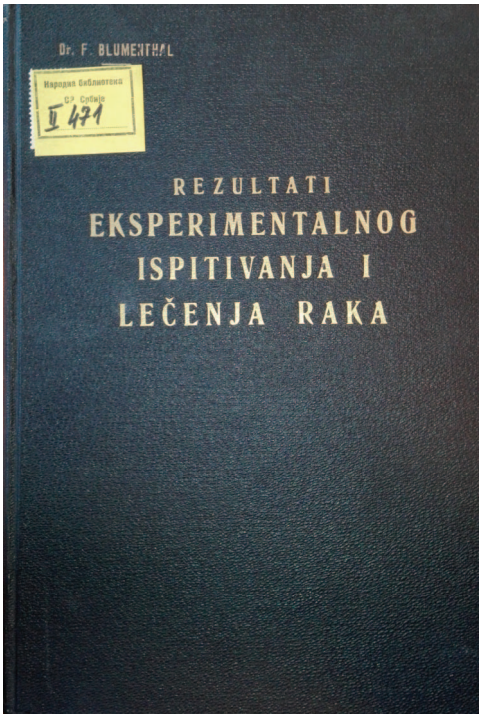

Fig. 2 – The book about the cancer research and therapy written by Ferdinand Blumenthal and translated into Serbian thanks to dr Lazar Stanojević.

Disputes and departure from Belgrade

At the same time, Blumenthal's stay in Belgrade was marked by various intense and increasingly frequent challenges. Although disputes in professional circles resulting from his role and position could in part be explained by competitiveness in the professional community, with an increasingly fascist-leaning public, more openly anti-Semitic challenges were raised in the discourse on the right. Soon after he arrived to Yugoslavia in autumn of 1933, *Lekar* (Doctor), a medical journal, published an article harshly criticizing the outpouring of "enthusiasm" for everything coming from abroad, and in particular that "various foreigners with exotic names had been enthusiastically accepted" and were seen as "messiahs" [23]. Soon after, the magazine *Život i rad* (Life and Work) published a defense of Blumenthal, advocating for the dismantlement of the local "pond-scum" and stating that Yugoslavia should be honored to host a scientist of such stature, while also pointing out that Blumenthal had been one of the top scientists in highly-competitive Germany. The piece published in *Lekar* made use of an anti-foreigner discourse that was a characteristic of the hidden anti-Semitism of the time, but these kinds of writings were not typical for the expert, professional publications. However, this kind of increasingly open anti-Semitic discourse was a characteristic of right-wing, nationalist publications like the newspaper *Narodna odbrana* (The People's Defense), which was produced by an organization of the same name, and one of the editors was Velibor Jonić, the future Minister of Education in Milan Nedić's collaborationist government. Starting in 1935, *Narodna odbrana* launched a campaign against Blumenthal, claiming that the University of Belgrade had lost its "national character" because preference was being given to the foreigners instead of to the "spiritual and moral values of our people." The newspaper lamented the fact that lectures at the University of Belgrade were held in German, and that students were allegedly forced into taking their exams in German to get better grades. Furthermore, while renouncing open support for Hitler's anti-Semitism in principle, the author of the article argued that "our university has welcomed with open arms every Jew the Führer of the Reich had banished from a strong, powerful, and cultured Germany" [13, 24, 25].

Blumenthal resigned from his professorship at the Faculty of Medicine on December 21, 1936, effective February 1, 1937. It is assumed that this decision had something to do with attempts to produce an experimental cancer treatment drug, which had garnered negative reactions within the medical profession [13]. At the time of his resignation, *Politika* dedicated a farewell article to him, expressing regret at the departure of such an "established scientist." The newspaper also pointed out that Blumenthal had been invited to Yugoslavia because there had been no such expert in experimental cancer research, and this was why he had been chosen to teach at the faculty, create a laboratory, establish an ambulatory unit for patient treatment, organize a system to fight cancer, train Yugoslav doctors, and so on. According to the newspaper, three years of work had not been enough to realize all these plans, primarily due to the prevailing conditions in medical circles. The newspaper also stated that, over the previous three years, Blumenthal had not only held his regular lectures at the faculty, but had also accepted all invitations from outside the university to hold lectures and training sessions. Blumenthal himself, however, claimed his departure was due to his three-year term as professor expiring and that his stay in Belgrade had been a pleasant one. He wished his colleagues and students success in their further endeavors and in continuing the work started by Đorđe Joannović, the greatest Yugoslav researcher in the field [26].

The anti-Semitic attacks on Blumenthal did not end there. At the end of 1936, when it was publicly announced that Blumenthal would be stepping down from his position at the university and leaving Yugoslavia, the anti-Semitic *Otadžbina* (Fatherland), the mouthpiece of Dimitrije Ljotić's fascist organization *Zbor*, bitterly attacked Blumenthal as "a son of the Chosen People" who had left Yugoslavia when they "had to pay taxes." In an article openly belittling Jewish persecution under the Third Reich in its description of Blumenthal's arrival in Yugoslavia, it was claimed that he had received "unlimited resources," which were, as the article put it, "the richest cream of our humble surroundings." The renowned scientist was dubbed a "fairytale-comedian," and his medicine "worthless garbage," from which he had earned over half a million dinars, while not paying a single dinar in taxes [27]. This article was just one of the characteristically anti-Semitic attacks in the press, in which the stereotype of Jews being associated with money and tax evasion was applied to actual circumstances in order to ethically discredit Blumenthal and the work he did in Belgrade.

Life after Yugoslavia

Blumenthal's life after leaving Yugoslavia became something of an odyssey. He first moved to Vienna, but after the German annexation of Austria in March 1938, he was arrested by the Gestapo and spent three months in prison. Afterwards he returned to Yugoslavia and made several unsuccessful attempts to emigrate to Great Britain. Then he accepted an invitation from Albania, and left Yugoslavia once again in January 1939, but his time in Albania was to be short-lived. Due to the looming threat of an Italian invasion and his experience with the Gestapo, he decided not to stay in Tirana, and left shortly after he arrived. He then went to Estonia, which was later occupied by the Soviet Red Army in 1940 after spheres of interest had been divided between Germany and the Soviet Union according to the 1939 Molotov–Ribbentrop Pact. Nevertheless, he remained there until Germany invaded the Soviet Union in June 1941. The Soviet authorities arranged for Blumenthal and his family to be transferred to Kazakhstan, but on July 5, 1941 their train was attacked by the German air force and Blumenthal was killed in the attack [4].

Conclusion

In the development of various branches of medicine in interwar Yugoslavia, the advancement of the Yugoslav, and

consequently, Serbian medical profession was contributed to the physicians who had been educated and built their professional careers outside Serbia, such as Đorđe Joannović and Richard Burian, Dean of the Faculty of Medicine. Within this context, Ferdinand Blumenthal's time in Belgrade should be considered a short historical episode that contributed to the improvement of cancer research and treatment in the Kingdom of Yugoslavia. The most important contribution Blumenthal gave to future generations of students in the Kingdom of Yugoslavia, however, was his book *Results of Experimental Cancer Research and Cancer Therapy* (published after his departure), which provided an overview of the current state of research in the field.

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b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure – **Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi – **Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhođane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp