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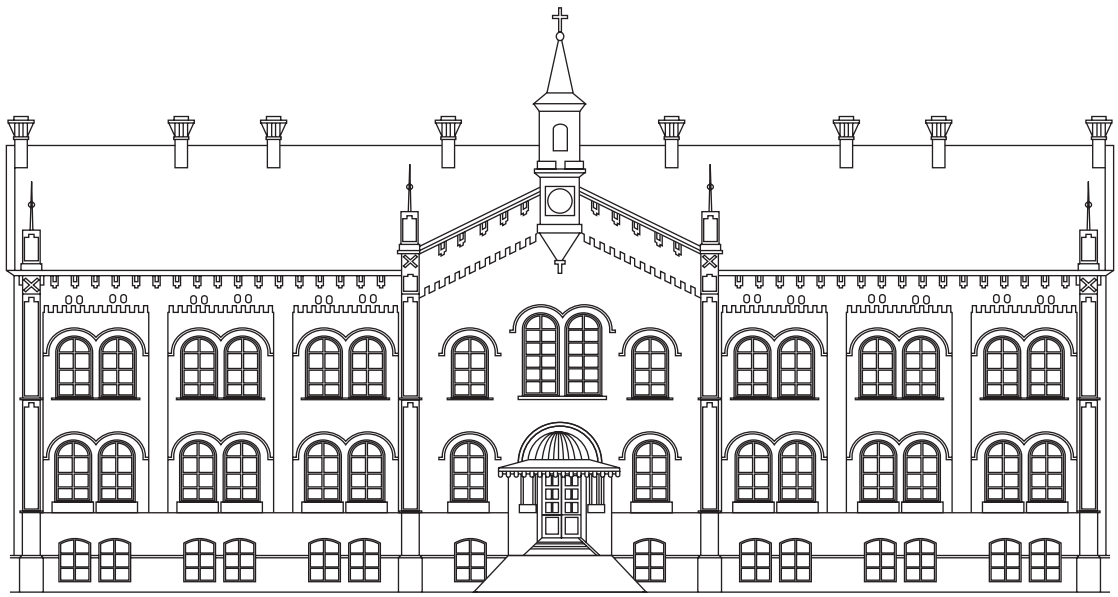

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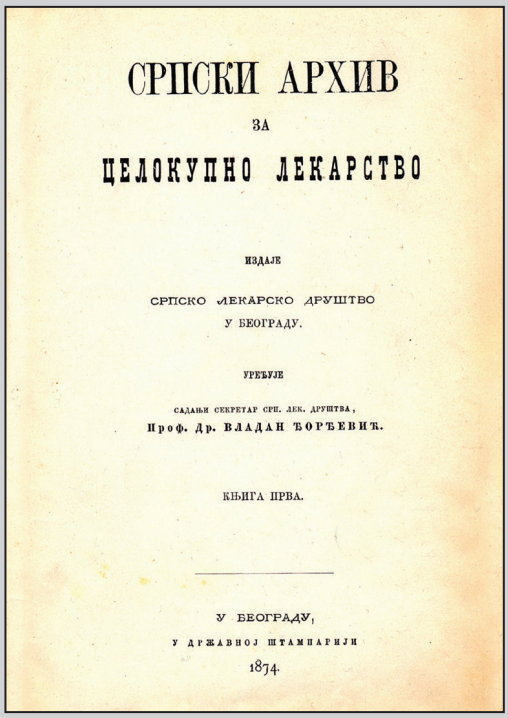

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ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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У БЕОГРАДУ.

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У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

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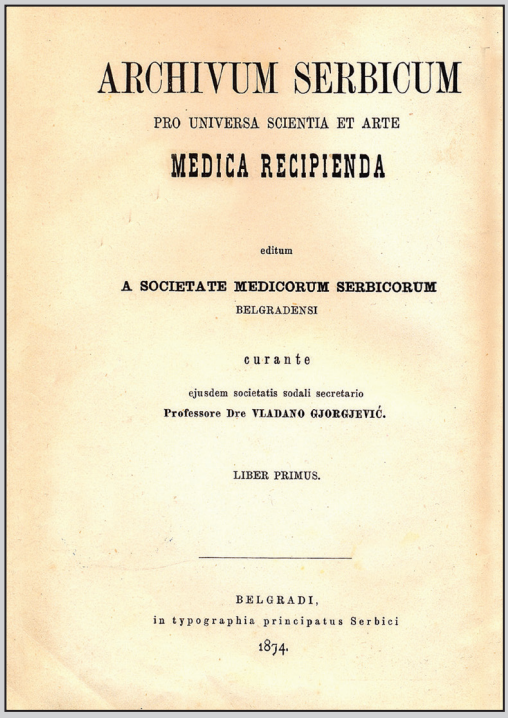

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in typographia principatus Serbici

1874.

The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of exercise during sport training on levels of salivary diagnostic markers

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SUMMARY

Introduction/Objective The aim of this study was to determine the changes in concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (CK), and salivary amylase in saliva samples collected before, immediately after, and 30 minutes after physical activity performed during basketball and mixed martial arts (MMA) training.

Methods Twenty-two athletes, 11 basketball players and 11 MMA fighters, 18 men and four women, aged 15–24 years, participated in the study. Saliva samples were collected using sterile saliva containers (Salivette®) from all participants before training (sample 1), immediately after (sample 2), and 30 minutes after training (sample 3). The levels of all investigated biomarkers were measured spectrophotometrically using a biochemical analyzer.

Results Statistically significant differences were present among samples 1, 2, and, 3 in the concentrations of urea, AST, and CK in samples collected from MMA fighters (Friedman test). Among three samples taken from basketball players, the significant differences were not observed for the analyzed parameters. When concentrations of all diagnostic markers were compared between basketball and MMA independently for samples 1, 2, and 3, statistically significant differences (Mann–Whitney U-test) existed in concentrations of urea, uric acid, proteins, and AST.

Conclusion Based on the results of the present study, the influence of the exercise on the levels of salivary diagnostic markers, such as urea, AST, and CK, is more evident during MMA than basketball training. Saliva composition of MMA fighters and basketball players differ in terms of levels of urea, uric acid, proteins, and AST.

Keywords: exercise; sport training; saliva diagnostic markers; basketball; mixed martial arts

INTRODUCTION

Physical activity, unique physiological stress, triggers a systematic series of neuroendocrine and immune events directed at bringing the system back to a state of homeostasis. Various physiological changes occurring in the human body during physical exercise contribute to accommodating the increase in physiological demands. Two major neuroendocrine stress response arms are the hypothalamic–pituitary–adrenal and the sympathetic–adreno–medullary axis, with both axes modulating the function of immune system [1, 2]. Immune and stress responses work together to combat exercise stress.

While blood samples have historically been used to measure numerous parameters, indicators of physiological and pathological processes in the organism, many of them could be analyzed in a much easier, less complex, and completely non-invasive way in the saliva samples [3, 4]. Saliva has been used to examine hydration, electrolyte status, stress, and immune responses during and after physical activity [5, 6, 7].

The salivary glands are under the control of the autonomic nervous system, parasympathetic cholinergic nerves and sympathetic adrenergic nerves. The type of activated autonomic receptor, salivary flow rate, and intensity and duration of stimulation to the glands can influence saliva composition. During prolonged and intense exercise, due to the increased sympathetic stimulation, reduced salivary flow rate is expected. Qualitative and quantitative changes are described by the increased concentration of total protein, cortisol, and hormones in saliva during the stressful period, as well as by the alterations in ionic composition of the saliva [5–8]. Immediately after intense physical activity, saliva remains viscous for some time, although the control of saliva secretion is no longer under sympathetic nervous system. These changes are primarily explained by mouth breathing during physical activity, dehydration of the organism, and increased secretion of salivary mucin [6–9]. After physical

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activity, the secretion of saliva is under control of the parasympathetic nervous system, which is active during the period of rest, and as these two systems have an antagonistic effect, increased secretion of the saliva, decreased protein concentration, and increased serosity are present.

Physical activity of any type may have implications for the immune system [10]. Changes in salivary secretion and composition and the alteration of the immune function that occur during intense physical activity may lead to the development of pathological changes. The occurrence of upper respiratory tract infections could be associated with systemic changes due to reduced immune response, as well as the lack of protective role of saliva in athletes due to reduced lubrication and IgA concentration [11, 12].

By measuring the levels of certain biomarkers in saliva samples, it is also possible to monitor changes in other organs whose functions may be affected by the intense physical activity. Among these biomarkers, the enzymes creatine kinase (CK), lactate dehydrogenase (LDH), or aspartate aminotransferase (AST) stand out as parameters that determine skeletal muscle injury and tissue damage in the muscles [13]. Previous studies reported changes in the levels of AST, CK, and LDH in saliva samples after intense exercise during different sports [14, 15].

The aim of this study was to determine the changes in concentrations of urea, creatinine, uric acid, proteins, AST, CK, and salivary amylase in saliva samples collected before, immediately after, and 30 minutes after physical activity performed during basketball and mixed martial arts (MMA) training. The null hypotheses were the following: (1) there are no statistically significant differences in the concentration of the mentioned biomarkers in saliva samples collected before, immediately after, and 30 minutes after training, regardless of the sport; (2) there are no statistically significant differences in the concentration of the biomarkers in saliva samples collected before, immediately after, and 30 minutes after intense physical activity, within each sport separately; and (3) there are no statistically significant differences in the concentration of the biomarkers in saliva samples collected before, immediately after, and 30 minutes after training, between the two sports, for all three samples separately.

METHODS

Participants

Twenty-two athletes, 11 basketball players and 11 MMA fighters, 18 men and four women, aged 15–24 years, participated in the study. Basketball players worked out five times a week, while MMA fighters worked out three times a week. The duration of the training for basketball players was two hours, while for MMA fighters it was one hour and 30 minutes. Distribution of the participants and their characteristics among the two groups is shown in Table 1.

Table 1. Distribution of the participants and their characteristics among the groups

Sport	n	Sex	Age (years) (Mean ± SD)	BMI (Mean ± SD)	Number of trainings per week	Training duration
Basketball	11	11 males	16.54 ± 1.03	21.7 ± 1.49	5	120 minutes
MMA	11	7 males 4 females	21.82 ± 1.83	23.75 ± 2.73	3	90 minutes
Total	22	18 males 4 females	19.18 ± 3.06	22.73 ± 2.38	-	-

MMA – mixed martial arts

All participants were given verbal and written explanation of the purpose and the protocol of the research previously approved by the institutional ethics committee, and their written informed consent for participation in the study was obtained. Main anamnestic data collected from the athletes indicated that all the participants were healthy, they did not suffer from any chronic disease, they had adequate oral hygiene habits, at least five balanced meals per day, and they all declared themselves as non-smokers. Also, basic dental examinations with a mirror and a probe were performed, and it was established that the participants did not have active pathological processes in the mouth.

Prior to sample collection, all the participants were familiarized with the experimental protocol, they were explained the rules of behavior, and shown the correct technique for saliva specimen collection. According to the instructions, they had to brush their teeth at least 30 minutes before their scheduled training and then refrain from taking food, caffein, alcohol, tobacco, chewing gums, juice, and energy drinks. Consuming water before and in-between sample collection was allowed.

Saliva samples

Saliva samples from all the participants were collected before training (sample 1), immediately after (sample 2), and 30 minutes after training (sample 3). For each sample, the athletes were asked to wash their hands and they were each given a sterile saliva container (Salivette®, Sarstedt, Germany) containing a sterile plain cotton swab. They were asked to open the lid of the container, take the cotton swab, and put it under the tongue for 3 minutes, while performing minimal orofacial movement. The cotton swabs were then placed back into Salivette® containers, the lid was closed, and each container was properly labeled. Saliva samples were immediately transferred to the laboratory for centrifugation (4200 × g, 10 minutes) and stored at the appropriate temperature (-20°C) before analyses were performed.

Saliva analyses

The levels of all investigated parameters (urea, creatinine, uric acid, proteins, AST, CK, and salivary amylase) were measured spectrophotometrically using a biochemical analyzer Rayto 1904-C (Rayto Life and Analytical Sciences Co., Ltd, Shenzhen, China). Appropriate reagents were used to obtain colored products (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany),

using the same methodology as for the serum measurements. The absorbances of the obtained colored compounds at certain wavelengths were measured, and then converted into quantitative concentration, mass concentration, or enzyme activity, depending on the biomarker.

Statistical analysis

The concentrations of the investigated biomarkers in the samples collected at three different time points relative to the physical activity (sample 1, sample 2, and sample 3) were statistically analyzed with Friedman test, regardless of the sport (all basketball and MMA samples together) and within each sport separately (basketball and MMA samples separately). Wilcoxon signed ranks test was used for post-hoc between-group comparisons when significant differences among three samples were detected by the Friedman test. Mann–Whitney U-test was used to assess the differences in the concentrations of the investigated parameters in samples 1, 2, and 3 independently, between two sports (basketball *vs*. MMA). The data were statistically analyzed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA), and significance level was set at $p < 0.05$ in all analyses.

RESULTS

Concentrations of urea, creatinine, uric acid, proteins, AST, CK, and salivary amylase in samples 1, 2, and 3 did not statistically differ among each other when all collected saliva samples were compared regardless of the sport (Friedman test, Table 2).

Table 2. Concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (CK), and salivary amylase in samples 1, 2, and 3, taken from all participants before, immediately after and 30 minutes after training, respectively

All participants (n = 22)	Sample 1 before training	Sample 2 immediately after training	Sample 3 30 minutes after training	p-value (Friedman test)
Urea (mmol/l)	4.98 ± 2.105	3.85 ± 2.398	4.89 ± 1.839	0.202
Creatinine (μmol/l)	23.03 ± 5.030	22.56 ± 4.465	23.49 ± 4.601	0.989
Uric acid (μmol/l)	212.23 ± 76.998	233.82 ± 92.464	246.04 ± 129.616	0.170
Proteins (g/l)	2.74 ± 2.008	6.19 ± 8.379	6.24 ± 8.268	0.795
AST (U/l)	23.27 ± 15.520	25.59 ± 18.477	20.64 ± 16.580	0.063
CK (U/l)	4.48 ± 5.577	4.64 ± 6.721	2.83 ± 3.339	0.086
Salivary amylase (U/ml)	148.68 ± 102.448	159.54 ± 118.983	169.18 ± 117.247	0.351

The results are presented as mean values ± standard deviation

Table 3. Concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (CK), and salivary amylase in samples 1, 2 and 3, taken from basketball players before, immediately after, and 30 minutes after training, respectively

Basketball players (n = 11)	Sample 1 before training	Sample 2 immediately after training	Sample 3 30 minutes after training	p-value (Friedman test)
Urea (mmol/l)	**4.99 ± 2.273**	**5.74 ± 1.374**	5.01 ± 1.126	0.103
Creatinine (μmol/l)	23.24 ± 6.331	22.37 ± 3.430	21.66 ± 3.663	0.643
Uric acid (μmol/l)	250.82 ± 82.014	268.54 ± 109.297	**290.36 ± 105.489**	0.441
Proteins (g/l)	**1.71 ± 1.181**	**1.16 ± 0.612**	**1.13 ± 0.683**	0.294
AST (U/l)	**19.73 ± 19.042**	**16 ± 13.550**	**12.64 ± 10.585**	0.461
CK (U/l)	4.59 ± 6.741	3.12 ± 2.982	3.76 ± 3.111	0.695
Salivary amylase (U/ml)	143.91 ± 94.159	147.82 ± 89.334	170.54 ± 128.543	0.234

The results are presented as mean values ± standard deviation; values in bold indicate the statistically significant difference with the corresponding values in the MMA samples shown in Table 4

Table 4. Concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (CK), and salivary amylase in samples 1, 2 and 3, taken from MMA fighters before, immediately after, and 30 minutes after training, respectively

MMA fighters (n = 11)	Sample 1 before training	Sample 2 immediately after training	Sample 3 30 minutes after training	p-value (Friedman test)
Urea (mmol/l)*	**2.97 ± 1.377^{B}**	**1.96 ± 1.536^{B}**	4.78 ± 2.410^{A}	0.020*
Creatinine (μmol/l)	22.82 ± 3.6	22.75 ± 5.480	25.32 ± 4.868	0.529
Uric acid (μmol/l)	173.64 ± 49.472	199.09 ± 57.923	**201.73 ± 140.826**	0.148
Proteins (g/l)	**3.78 ± 2.171**	**11.22 ± 9.564**	**11.36 ± 9.245**	0.175
AST (U/l)*	**26.82 ± 10.75^{AB}**	**35.18 ± 18.192^{A}**	**28.64 ± 18.013^{B}**	0.026*
CK (U/l)*	4.37 ± 44.454^{AB}	6.15 ± 8.994^{A}	1.90 ± 3.439^{B}	0.021*
Salivary amylase (U/ml)	153.45 ± 114.563	171.27 ± 146.447	167.82 ± 111.091	0.534

The results are presented as mean values ± standard deviation;
*presence of statistically significant difference; different superscript letters indicate statistically significant difference among the values presented in the same row;
values in bold indicate the statistically significant difference with the corresponding values in the basketball samples shown in Table 3

When concentrations of examined parameters were statistically analyzed among samples 1, 2, and 3 separately for basketball and MMA, there were no significant differences for any of the parameters in the samples collected from basketball players (Friedman test, Table 3), whereas statistically significant differences were present in the concentrations of urea, AST, and CK in the samples collected from MMA fighters (Friedman test, Table 4). Between-group comparisons revealed that the concentration of urea was significantly higher in sample 3 than in samples 1 and 2, while concentrations of AST and CK were significantly different only between samples 2 and 3 (Wilcoxon signed ranks test, Table 4).

Additionally, concentrations of all examined parameters were compared between basketball and MMA (Mann–Whitney U-test) independently for samples 1, 2, and 3. In samples taken before training (sample 1) statistically significant differences existed in concentrations of urea ($p = 0.010$), proteins ($p = 0.023$) and AST ($p = 0.047$) between basketball and MMA samples. Immediately after training (sample 2), statistically significant differences were present in concentrations of urea ($p = 0.000$), proteins ($p = 0.019$), and AST ($p = 0.005$) between basketball and MMA samples. In samples taken 30 minutes after training (sample 3), statistically significant differences were present in concentrations of uric acid ($p = 0.013$), proteins

($p = 0.002$), and AST ($p = 0.002$). Concentrations of other examined parameters did not significantly differ between the two sports.

DISCUSSION

A lack of statistically significant differences in the concentration of any of the parameters among saliva samples collected before, immediately after, and 30 minutes after training, when all participants were analyzed, regardless of the sport, led to the acceptance of the first null hypothesis. The second null hypothesis was accepted for basketball, as no statistically significant difference was detected in the concentration of any of the parameters among the three saliva samples, while the second null hypothesis was rejected for MMA, where statistically significant differences were present in the concentrations of urea, AST, and CK among samples 1, 2, and 3. The third null hypothesis was also rejected, since significant differences existed in the concentrations of urea, uric acid, proteins, and AST between basketball and MMA samples.

In the present study, there were no statistically significant differences in the concentrations of salivary amylase and proteins in the total sample, nor when the two sports were analyzed separately, which is in line with some previous findings [9]. Nevertheless, opposing results have also been reported [16]. Higher levels of these biomarkers were expected after exercise, considering that their secretion is predominantly controlled by the sympathetic nervous system activated in stress [5, 6]. It could be assumed that sport trainings in this study had no significant effect on the protein and salivary amylase concentrations because the exercise intensity was below the anaerobic threshold, when salivary amylase and proteins were proved to increase [17]. It should be noted that the level of proteins in the MMA group was significantly higher than that in the basketball group in all three samples, suggesting that MMA training is probably closer to the anaerobic threshold level.

Higher levels of serum enzymes CK, AST, and LDH could be used as indirect markers of muscle damage, and in apparently healthy subjects they may be correlated with physical training status [13, 18, 19]. The results of this study showed that in the MMA group AST and CK salivary levels increased immediately after training and returned to their initial values in samples taken 30 minutes after training, suggesting that this change is transitory.

Interestingly, the levels of salivary AST were significantly higher in all three MMA saliva samples than in the basketball samples. Based on this finding it could be assumed that MMA fighters are more likely to have permanent increase of AST in saliva. Furthermore, analyses of key salivary electrolytes, stress and immune markers, and muscle damage markers in the saliva samples showed that male and female organisms have different response to exercise stress [15, 20]. A greater response to exercise stress was noticed in females, as there were significant increases in osmolality, salivary amylase activity, and secretion rate and salivary IgA secretion rate, whereas such differences between rest and exercise were not present for any salivary analytes in males [20]. Also, the three enzymes indicating muscle damage (AST, LDH, and CK) showed different responses in men and women playing rugby, with AST showing the most significant variations, which were more pronounced in men than in women [15]. In the present study, around 18% were females (four out of 22) in the total sample, and all of them were in the MMA group, where they represented around 36% (four out of 11), while in the basketball group all participants were males, which could explain the differences in the results between the studies. Further research should focus on the investigation of the influence of sex and type of sport on these salivary biomarkers at rest and during exercise.

Differences in the levels of biomarkers between basketball and MMA samples observed in the present study may be due to several other reasons, such as younger age, lower BMI, more frequent and longer trainings of basketball players than those of MMA fighters, as well as due to basic differences in these two sports. Basketball is a team sport with a ball, characterized mainly by running and shooting, while MMA is a combat sport, with two distinct categories – grappling and striking. Therefore, these two sports develop different physical and physiological profiles, in terms of aerobic and anaerobic capacities, strength, kinematic, and neuromuscular variables [21, 22].

It is well-known that saliva also contains urea, creatinine, and other markers of renal function. Studies have shown that the salivary concentrations of these markers are useful for the assessment of kidney function, and one study investigated them as possible markers for periodontal disease [23, 24]. To the best of the authors' knowledge, the assessment of salivary concentration of these parameters has not been used in relation to the physical activity, sport, and exercise. While the levels of creatinine did not show significant changes among the collected samples and groups, concentration of urea in MMA samples 1 and 2 was significantly lower than in sample 3, and it was also lower in MMA samples 1 and 2 compared to that of basketball samples 1 and 2. These differences could probably be associated with urea excretion through sweat and individual characteristics that affect sweating.

It was expected that exercise would cause an increase in the concentration of uric acid, a salivary antioxidant, as antioxidant responses are promoted by physical activity and the antioxidant profile of saliva samples showed to be very similar to that of plasma [25]. The results of this study showed that concentration of uric acid was higher in samples taken after training than in samples taken before training, but the difference was not statistically significant in any of the analyses. However, a significantly higher concentration of uric acid 30 minutes after training observed in the saliva of basketball players than in that of MMA fighters suggests that activities similar to basketball may lead to more pronounced antioxidant responses and consequently to the physiological processes related to redox. It may also be because basketball players trained more often, they were probably in better physical shape, and they were younger than MMA fighters that participated in this study.

Lack of significant difference in the concentration of some markers before and after training could be explained by an early activation of stress response that is not directly related to the physical activity during training, but to the research itself, i.e., excitement or fear of the unknown which participants may have had upon entering the study. On the other hand, sample 1 was taken prior to training, but after participants' arrival at the training site, hence one should have in mind that they had certain activity (e.g., walking, fast walking, public transportation) during arrival, so sample 1 could not be entirely considered a sample at complete rest.

CONCLUSION

In conclusion, the influence of the exercise on the levels of salivary diagnostic markers, such as urea, AST and CK, is more evident during MMA than basketball training. Saliva composition of MMA fighters and basketball players differ in terms of levels of urea, uric acid, proteins, and AST composition. Saliva may be an alternative and noninvasive tool in sports medicine for the study of salivary proteins, stress and immune markers, antioxidants, and muscle damage enzymes in different physical exercise protocols.

Conflict of interest: None declared.

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Утицај физичке активности током спортског тренинга на ниво дијагностичких маркера пљувачке

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САЖЕТАК

Увод/Циљ Циљ ове студије био је да се утврде промене у концентрацији урее, креатинина, мокраћне киселине, протеина, аспартат-аминотрансферазе (АСТ), креатин-киназе (КК) и саливарне амилазе у узорцима пљувачке прикупљеним пре, непосредно након и 30 минута након физичке активности која се изводила током тренинга кошарке и мешовитих борилачких вештина (ММА).

Методе У истраживању је учествовало двадесет двоје спортиста, 11 кошаркаша и 11 ММА бораца, 18 мушкараца и четири жене, старости 15–24 године. Узорци пљувачке сакупљани су у стерилне епрувете за пљувачку (*Salivette*®) од свих учесника пре тренинга (узорак 1), непосредно након (узорак 2) и 30 минута након тренинга (узорак 3). Нивои свих испитиваних биомаркера измерени су спектрофотометријском методом у биохемијском анализатору.

Резултати Статистички значајне разлике биле су присутне међу узорцима 1, 2 и 3 у концентрацијама урее, АСТ и КК у узорцима прикупљеним од ММА бораца (Фридманов тест). Међу три узорка узета од кошаркаша нису уочене статистички значајне разлике у анализираним параметрима. Када су упоређиване концентрације свих дијагностичких маркера између кошарке и ММА, независно за узорке 1, 2 и 3, постојале су статистички значајне разлике (Ман–Витни *U* тест) у концентрацијама урее, мокраћне киселине, протеина и АСТ.

Закључак На основу резултата ове студије, утицај физичке активности на нивое дијагностичких маркера пљувачке, као што су уреа, АСТ и КК, израженији је код ММА у односу на кошаркашки тренинг. Састав пљувачке ММА бораца и кошаркаша разликује се у погледу нивоа урее, мокраћне киселине, протеина и АСТ.

Кључне речи: физичка активност; спортски тренинг; дијагностички маркери пљувачке; кошарка; мешовите борилачке вештине

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Impact of ketamine on spontaneous coordinate activity and short memory behavior in rodents' chronic unpredictable stress model

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SUMMARY

Introduction/Objective This research aims to evaluate the impact of chronic stress on behavioral effects of ketamine, which are still not sufficiently clear.

Methods Wistar male rats aged five weeks were used in the experiment. The animals were divided into two equal groups: control and experimental. After being exposed to a chronic unpredictable stress paradigm for 42 days, experimental rats received a single injection of ketamine (10 mg/kg; day 45) as did the control group. The impact of ketamine was assessed using behavioral tests, spontaneous coordinate activity, and water maze tests for the evaluation of short-term memory.

Results The experimental group rats showed less spontaneous motoric activity than before ketamine application. Statistical significance was shown in gaining weight after time of ketamine application in the control group, as well as in the experimental group, where they showed weight loss during stress paradigm and then increased their weight after ketamine application. There was no statistical significance in speed measurements in either group, showing no effects on short-term memory behavior.

Conclusion These findings show that ketamine in a single subanesthetic dose has antidepressant and anxiolytic-like effects in male rats exposed to chronic unpredictable stress paradigm.

Keywords: Wistar rat; chronic unpredictable stress paradigm; ketamine; behavior

INTRODUCTION

Anxiety presents a normal reaction to various stressful events and is most often very useful in some situations, by helping one prepare for potential danger and inducing one's adequate reaction. Anxiety disorders refer to anticipation of future concerns and doubts, fear and avoidance. They also present the most common group of mental disorders generally nowadays [1]. Drugs that affect the serotonergic and GABAergic neurotransmission are often used in treatment of stress disorders but also show some limitations in their use, aiming further investigations in other direction [2]. A great potential of different negative glutamate transmission modulators has been shown as a result of many studies [3, 4]. Ketamine presents a dissociative anesthetic, non-competitive N-methyl-D-aspartate receptor antagonist with rapid and sustained anxiolytic and antidepressant effects manifested in clinical and preclinical studies lasting for a couple of weeks, which means that probably even a single dose of ketamine might have beneficial effects on anxiety conditions [5]. There is significant discrepancy among preclinical studies related to anxiolytic effects of ketamine. There are also different profiles of ketamine that make an impact in animal tests concerning anxiety/stress/fear, which much depend on the experimental paradigm, schedule of ketamine application, doses, and tested animals. Initially proposed as a depression model, chronic unpredictable stress paradigm (CUS) is a widely used paradigm in investigating stress disorders in preclinical research in rodents. It comprises continuous and consecutive exposures to variable, unpredictable, and aversive stressors lasting for weeks [6]. Many already conducted studies showed great similarity of paradigm variables to chronic stressful conditions of human life as well as decreased locomotor and exploratory activity and impaired learning and memory as one of the signs of anhedonia after CUS paradigm was completed [7].

This research aims to evaluate the impact of chronic stress on behavioral effects of ketamine. The objective of this study was to investigate the impact of chronic stress on tested animal behavior after ketamine was applied and whether ketamine could improve anxiety-like behaviors seen in rats exposed to chronic unpredictable stressors and to determine possible variations.

METHODS

Wistar male rats (250–300 grams; Faculty of Medicine, University of Belgrade), aged five weeks (approximates time of adolescence in

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humans), were used at the beginning of the experiment. They were allowed one week acclimatization period before unpredictable stress exposure. There were 16 rats in total, kept in Makrolon cages, two animals per cage, and fed *ad libitum* with a full rat mixture formula (Veterinary Institute, Subotica); water used for the animals was from the Belgrade water supply; room temperature was 22 ± 2°C, following the 12 to 12 day and night regime. At the beginning of the experiment, the animals were divided into two equal groups: control and experimental, each containing eight rats. The control group was kept and fed as previously specified. The experimental group was exposed to continuous stress during 42 days according to the following schedule: on day one, the animals were transferred from one cage into the other, so that the pairs from the beginning of the experiment are separated; on day two, the animals were exposed to 24-hour light; on day three, they were retransferred to separate the previously formed pairs; on day four, the animals were subjected to tail clipping by taking the tip of the tail with a clamp, lifting the animal 20 cm above the cage where it was kept for 15 seconds and returned back to the cage; on day five, the animals were retransferred to separate the previously formed pairs, and on day 6, they were deprived of food and water during 24 hours. That cycle was repeated until day 42 from the beginning of the experiment. [8, 9]. After 42 days from the beginning of the experiment, the animals were exposed to behavioral tests as follows: observation of spontaneous activity in the new space and testing short-term memory in a water pool without any previous treatment, following intraperitoneal administration of 10 mg/kg ketamine hydrochloride and 20 minutes after ketamine was applied, observation of spontaneous activity and short-term memory testing in a water pool. The control group was allowed usual animal activities and was subjected to the same tests, and ketamine was administered in the same dose. After testing, all animals were tested for glucose levels. The weighing of animals was performed three times. The first weighing was during the stress paradigm, the second one was performed seven days after, before ketamine application, and third weighing was also six days after the second weighing.

All experiments were carried out according to the NIH Guide for Care and Use of Laboratory Animals and were approved by the Ethics Committee of the University of Belgrade (permit number 513/1, 27.01.2020).

Spontaneous activity

Spontaneous activity of the rats was measured on the 43rd day using an Ugo Basile Activity Cage 7401 device. The rats were placed into the device individually and kept there for two hours, during which time the number of spontaneous movements was measured at five-minute intervals.

Short-term memory

Short-term memory and spatial navigation learning were tested on the 44th day using a 25°C, circular, 40-cm deep water pool; in one quadrant of the pool there was a rectangular 15 × 15 cm "island" with surface 1 cm below the surface of the water. The animals were prepared the previous day by being placed into the water and slowly directed to swim towards the "island." If a rat did not locate the platform after 90 seconds, it would be guided to the platform and allowed to remain on the platform for 20 seconds to recognize the location. The rats received three such consecutive trials on the day before the testing day with an intertrial interval of 30 seconds. The water was changed each day. The escape latency time for the rat to locate and climb onto the platform was observed and recorded. For each trial, the rats were allowed to search for the hidden platform for a 90-second period. The day after the preparation, the procedure was repeated, the time elapsed from placing an animal into the water until it found the "island" and climbed onto it was measured by a chronometer. On the 45th day, the same procedure was repeated, but this time 20 minutes after ketamine administration. This test was used to measure spatial navigation learning and short memory in rats. Both groups of rats were trained for one day, and the swimming test was performed as described previously [10].

Statistical analysis

All observed data have normal distribution, so that the following parameter tests were performed: the parameter paired t-test within groups and the parameter matched t-test for comparing pair times. In addition, Pearson's correlation was used in order to establish the degree of linear connection.

RESULTS

Comparison of successive times in spontaneous activity measurement in the experimental group before and after ketamine application was performed in this experiment. The same procedure was performed within the control group. The differences between time parameters between the experimental and the control group were not calculated as we aimed to examine the behavior in each group independently, to show the effects of ketamine on the animals' behavior.

Before ketamine application in the experimental group, the results showed statistical significance in time windows between the fifth and the 10th minute, the 10th and the 15th, the 15th and the 20th, and the 55th and the 60th minute. In the control group, the statistical significance was shown in successive time windows between the 15th and the 20th minute and the 50th and the 55th minute (Table 1 and Figure 1).

After ketamine application in the experimental group, the statistical significance was shown in time windows between the 15th and the 20th minute and the 25th and the 30th minute. In the control group, the statistical significance was shown between the 10th and the 15th minute and the 35th and the 40th minute (Table 2 and Figure 2).

Table 1. Succeeded time comparison in control and stress groups before treatment

Paired samples statistics		Control group			Stress group		
		Mean	SD	p	Mean	SD	p
Pair 1	Time5	144.67	82.641	0.535	241.38	43.041	**0.006**
	Time10	126.5	42.627		164.38	44.397	
Pair 2	Time10	126.5	42.627	0.235	164.38	44.397	**0.014**
	Time15	97.83	16.278		118.63	49.753	
Pair 3	Time15	97.83	16.278	**0.000**	118.63	49.753	**0.015**
	Time20	73.17	17.429		79	35.505	
Pair 4	Time20	73.17	17.429	0.336	79	35.505	0.698
	Time25	63	21.457		76.88	39.948	
Pair 5	Time25	63	21.457	0.809	76.88	39.948	0.179
	Time30	57.67	61.617		56.75	29.085	
Pair 6	Time30	57.67	61.617	0.850	56.75	29.085	0.130
	Time35	62.17	33.772		42	22.552	
Pair 7	Time35	62.17	33.772	0.563	42	22.552	0.570
	Time40	54.17	39.686		36.25	20.852	
Pair 8	Time40	54.17	39.686	0.116	36.25	20.852	0.623
	Time45	33.67	24.476		42	19.198	
Pair 9	Time45	33.67	24.476	0.372	42	19.198	0.358
	Time50	48	20.794		33.5	24.378	
Pair 10	Time50	48	20.794	**0.008**	33.5	24.378	0.292
	Time55	24.5	18.982		25.75	18.352	
Pair 11	Time55	24.5	18.982	0.826	25.75	18.352	**0.025**
	Time60	22.83	16.29		48.5	28.122	

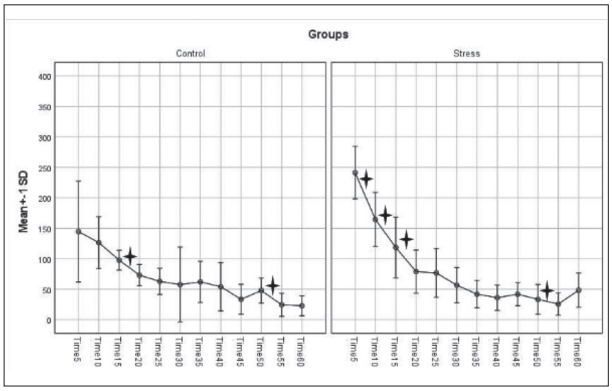

Figure 1. Successive times in spontaneous activity measurement in experimental group before ketamine application

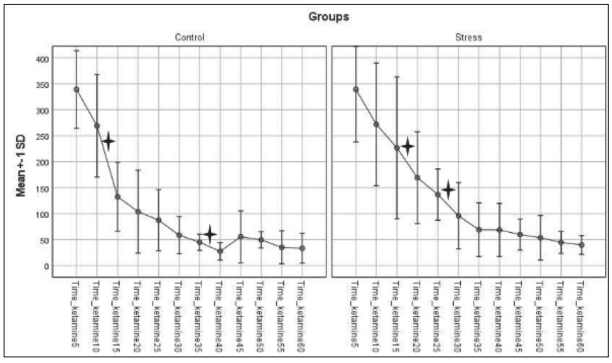

Figure 2. Successive times in spontaneous activity measurement in experimental group after ketamine application

After ketamine was applied, the experimental group showed less spontaneous motor activity than before ketamine was applied.

The control group showed weight gain after ketamine was applied and experimental group showed weight loss when stressed, during CUS paradigm, but also weight gain after ketamine was applied (Figure 4 and Table 3).

There was no statistical significance in speed measurement in both groups.

The glucose level of the control group was elevated as late as 30 minutes after the baseline and then returned to normal. As for the stress group, it can be seen that the glucose level rise was abrupt and differed compared to the baseline and then dropped after 30 minutes. Statistically significant rise/drop was observed almost at all measured times (Figure 3 and Table 3).

Table 2. Succeeded time comparison in control and stress groups after treatment of ketamine

Paired samples statistics		Control group			Stress group		
		Mean	SD	p	Mean	SD	p
Pair 1	Time_ketamine5	339	74.825	0.068	339.25	101.064	0.099
	Time_ketamine10	269.17	98.493		271.88	118.082	
Pair 2	Time_ketamine10	269.17	98.493	**0.005**	271.88	118.082	0.186
	Time_ketamine15	132.5	66.443		226.75	136.581	
Pair 3	Time_ketamine15	132.5	66.443	0.074	226.75	136.581	**0.056**
	Time_ketamine20	104	79.815		169.25	88.286	
Pair 4	Time_ketamine20	104	79.815	0.270	169.25	88.286	0.271
	Time_ketamine25	87.5	58.76		136.75	49.204	
Pair 5	Time_ketamine25	87.5	58.76	0.271	136.75	49.204	**0.014**
	Time_ketamine30	58.67	35.943		96	63.933	
Pair 6	Time_ketamine30	58.67	35.943	0.407	96	63.933	0.102
	Time_ketamine35	45	15.427		69	51.758	
Pair 7	Time_ketamine35	45	15.427	**0.012**	69	51.758	0.980
	Time_ketamine40	27.5	16.814		68.63	51.264	
Pair 8	Time_ketamine40	27.5	16.814	0.189	68.63	51.264	0.477
	Time_ketamine45	55.5	50.007		59.75	29.793	
Pair 9	Time_ketamine45	55.5	50.007	0.800	59.75	29.793	0.561
	Time_ketamine50	49.67	15.565		53.63	42.915	
Pair 10	Time_ketamine50	49.67	15.565	0.286	53.63	42.915	0.625
	Time_ketamine55	35.17	31.884		44.75	21.346	
Pair 11	Time_ketamine55	35.17	31.884	0.937	44.75	21.346	0.693
	Time_ketamine60	33.5	28.829		39.75	18.077	

DISCUSSION

We investigated the effects of ketamine on spontaneous locomotor activity and short memory in rats within the CUS model. Spontaneous locomotor activity was measured in an activity cage; recording values which indicate pulses were recorded by the apparatus as the stainless bars tilted in response to animal movements, and activity of each rat was automatically recorded for consecutive five minutes. Our results showed an increase of spontaneous activity in both experimental and control groups of animals before ketamine was applied, in the time window between the 15th and the 20th minute and the 55th and the 60th minute of measurement, but at the beginning,

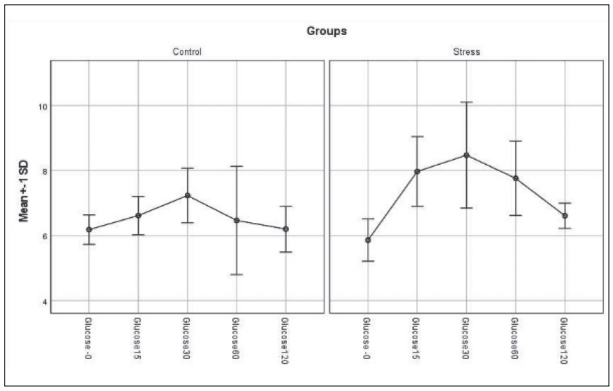

Figure 3. Glucose concentrations in the experimental and the control group

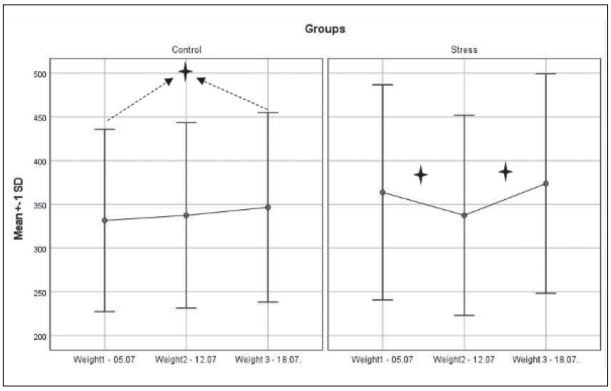

Figure 4. Weight changes in experimental and control groups in three time-point measurements

Table 3. Succeeded time comparison in control and stress groups for weight, speed, and glucose

Paired samples statistics		Control group			Stress group		
		Mean	SD	p	Mean	SD	p
Pair 1	Weight 1	331.667	104.195	0.201	363.75	122.962	**0.016**
	Weight 2	337.500	105.818		337.5	114.268	
Pair 2	Weight 1	331.667	104.195	**0.030**	363.75	122.962	0.353
	Weight 3	346.667	108.382		373.75	125.235	
Pair 3	Weight 2	337.5	105.818	**0.006**	337.5	114.268	**0.000**
	Weight 3	346.667	108.382		373.75	125.235	
Pair 1	Speed	15.5	9.203	0.177	9.5	8	0.779
	Speed ket.	8.5	4.231		8.625	4.868	
Pair 1	Glucose0	6.183	0.454	**0.056**	5.863	0.652	**0.001**
	Glucose15	6.617	0.591		7.975	1.071	
Pair 2	Glucose0	6.183	0.454	**0.027**	5.863	0.652	**0.006**
	Glucose30	7.233	0.838		8.475	1.627	
Pair 3	Glucose0	6.183	0.454	0.689	5.863	0.652	**0.001**
	Glucose60	6.467	1.665		7.763	1.143	
Pair 4	Glucose0	6.183	0.454	0.955	5.863	0.652	**0.026**
	Glucose120	6.2	0.704		6.613	0.387	
Pair 5	Glucose15	6.617	0.591	0.135	7.975	1.071	0.281
	Glucose30	7.233	0.838		8.475	1.627	
Pair 6	Glucose15	6.617	0.591	0.843	7.975	1.071	0.720
	Glucose60	6.467	1.665		7.763	1.143	
Pair 7	Glucose15	6.617	0.591	0.298	7.975	1.071	**0.013**
	Glucose120	6.2	0.704		6.613	0.387	
Pair 8	Glucose30	7.233	0.838	0.158	8.475	1.627	0.392
	Glucose60	6.467	1.665		7.763	1.143	
Pair 9	Glucose30	7.233	0.838	0.135	8.475	1.627	**0.020**
	Glucose120	6.2	0.704		6.613	0.387	
Pair 10	Glucose60	6.467	1.665	0.783	7.763	1.143	**0.018**
	Glucose120	6.2	0.704		6.613	0.387	

only the experimental group showed activity. These results showed immediate effects of CUS in the group of experimental animals as increased locomotor activity and as a result of anticipating pain and stress, which is in consistency with previously conducted investigations [11, 12, 13].

The experimental group rats showed less spontaneous motoric activity than before ketamine application, which shows longer term effects of ketamine administration and its anxiolytic effects as well, which was also shown in a study conducted by Bates and Trujillo [14], who also showed that repeated ketamine application might lead to addiction, with no statistical significance in cognitive deficits, memory, and spatial learning, which is in consistency with our findings related to the speed of swimming of animals and short-term memory, where we also found no statistical significance [14]. The inability of low ketamine dose to affect memory can be due to the short half-life of ketamine. At lower, subanesthetic doses, ketamine is able to mimic the effects of an antidepressant [15]. We administered ketamine at a single dose of 10 mg/kg, which was chosen as it is regarded to represent a recreational dose for use in rodents with LD50 of 600 mg/kg at four-hour intervals and was consistent with dosages reported in literature shown to be subanesthetic and primarily anxiolytic and antidepressant in rodents [16]. Noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonists produce antidepressant effects after a single administration, which was shown at the forced swimming test and the tail suspension test. Research that included ketamine's antidepressant effects after acute single application showed that acute treatment with a noncompetitive NMDA channel blocker tends to improve depressive and anxiolytic behaviors induced by chronic stress [17]. Opposite to this, animals that were repeatedly administered ketamine demonstrated locomotor sensitization and addiction [18]. Previous research investigating the effectiveness of noncompetitive NMDA receptor antagonists has revealed inconsistent results. Recent research, however, has been providing robust evidence for ketamine's anxiolytic effects. In a study that investigated acute effects of NMDA receptor blockade with ketamine in an animal model of fear-conditioning affecting frequency and duration of freezing as well as associated neural changes in the subcortical structures, the results indicated that ketamine normalized stress-related depressive behaviors in areas associated with fear and anxiety [19]. Clinical use of ketamine, esketamine, has gained broad attention because of its rapid therapeutic effects, as well as effects that last for a significant amount of time after a single dose in treatment of depression-resistant patients [20]. As previously described, our investigation showed statistical significance related to weight was shown in the experimental group after stress paradigm (weight loss), after ketamine

application (weight gain) and between the first and the third weight measurement there were of no statistical significance. Our experimental group of animals showed weight loss due to the CUS paradigm, which confirms that these rats were in a state of anhedonia, one of the major signs of depression. Previous studies showed that chronic stress in the rodent stress animal model induces specific patterns of behavioral activity that indicate depression or anxiety, like anhedonia and loss of interest when exposed to behavioral tests [21]. The previous study of Cox et al. [22] also showed that the experimental group had slower weight gain even if the CUS model they used did not include food and water deprivation and increased locomotor activity noticed in behavior tests is proved not to be linked to this weight loss phenomenon. The weight measurement was done during the CUS, then after ketamine application (seven days after), and finally six days after the second measurement. There was no difference between the first and the third measurement, as experimental animals who were under stress events felt anhedonia and ate less, while after some time ketamine effects showed in weight gain. Even though stress enhances the response to insulin, our results showed that glucose level was significantly different and slower in its metabolism in the experimental group compared to the control group where its levels returned to normal after an hour. In the experimental group glucose levels remained higher than normal after the same period of time. Previous studies showed increased blood levels of glucose in rodents that were exposed to CUS and with ketamine application where short-term effect of ketamine was shown on regulation of body weight and food intake [23].

CONCLUSION

Our results clearly suggest that ketamine has anxiolytic properties on behavior at doses that do not produce short memory impairment but improve locomotor activity and weight gain. The anxiolytic effect of ketamine may be related to several neuromediator systems that are known to be involved in neuropharmacology of anxiety, such as serotonergic, glutamatergic, and GABAergic. Further research should elucidate the neuronal mechanisms that underlie specific differences in response to ketamine and highly specific mechanisms responsible for lasting, non-addictive effects on behavior.

Conflict of interest: None declared.

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Ефекти кетамина на спонтану координатну активност и краткорочну меморију у хроничном непредвидивом моделу стреса код глодара

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САЖЕТАК

Увод/Циљ Ово истраживање има за циљ да процени ефекте хроничног стреса на бихејвиоралне ефекте кетамина, који још увек нису довољно разјашњени.

Методе У експерименту су коришћени мушки пацови соја Вистар стари пет недеља. Животиње су подељене у две једнаке групе: контролну и експерименталну. Након што су били изложени парадигми хроничног непредвидивог стреса током 42 дана, експериментални пацови су примили једну инјекцију кетамина (10 *mg/kg*; 45. дан) као и контролна група. Утицај кетамина процењен је помоћу тестова понашања, спонтане координатне активности и тестова воденог лавиринта за процену краткорочног памћења.

Резултати Експериментални пацови су показали мање спонтане моторичке активности пре апликације кетамина. Повећање тежине је показано након апликације кетамина у контролној групи. У експерименталној групи је показан губитак тежине након парадигме стреса, а затим је показано повећање тежине након апликације кетамина. Није било статистичке значајности у мерењу брзине у обе групе, што указује да није било ефекта у краткорочној меморији.

Закључак Ови налази показују да једнократно примењен кетамин у субанестетичкој дози поседује антидепресивне и анксиолитичке ефекте код пацова мужјака изложеним парадигми хроничног непредвидивог стреса.

Кључне речи: Вистар пацов; парадигма хроничног непредвидивог стреса; кетамин; понашање

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The importance of laparoscopic surgery for early postoperative course in patients with colorectal carcinoma

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SUMMARY
Introduction/Objective The aim of our study was to compare early postoperative recovery in patients operated on using laparoscopically assisted and open method in colorectal carcinoma surgery.
Methods The study involved 60 patients, divided into two groups of 30 patients each, treated with open or laparoscopically assisted colorectal surgery. Three groups of factors were collected and analyzed for all the patients. The first group of factors were as follows: age, sex, the American Society of Anesthesiologists score, preoperative hemoglobin, localization. The second group of factors were the following: intraoperative complications, the duration of operations, blood and blood derivatives' compensation. The third group were as follows: complications, length of stay in intensive care, rate of peristaltic establishment, and the time needed for unobstructed oral intake, number of hospitalization days, analgesic use, and verticalization time.
Results The patients who underwent laparoscopically assisted surgery showed significant advantages in the early postoperative recovery compared with those who underwent open surgery, in terms of the number of postoperative days of hospitalization ($p < 0.001$), the duration of the operation ($p < 0.001$), the day of establishment of peristalsis ($p = 0.009$), and the day of establishment of unobstructed oral intake ($p < 0.001$), the time of verticalization of the patients ($p = 0.001$), the use of analgesics ($p < 0.001$).
Conclusion Laparoscopically assisted surgery has an advantage over open surgery colorectal cancer, as regards of early postoperative recovery of the patient.
Keywords: laparoscopic colorectal surgery; open colorectal surgery; colorectal cancer

INTRODUCTION

Colorectal cancer is the third most common cancer in men (746,000 patients per year, 10% of total cancer patients) and the second most prevalent cancer in women (614,000 patients per year, 9.2% of total cancer patients). It is represented in 8.5% of all patients with malignant tumors in the world [1]. With continuous improvement of modern medicine and technology, the aims are set to faster recovery time, as well as the reduction of postoperative morbidity and mortality.

Laparoscopic colorectal surgery has been routinely performed by the surgeons of the Department for General Surgery, Zemun Clinical Hospital Center since 2013. The aim of our study was to compare early postoperative recovery in patients treated with laparoscopically assisted and classical, open method in colorectal cancer surgery.

METHODS

The study was performed as a clinical retrospective study. It included 60 patients who underwent elective laparoscopically assisted or open colorectal surgery at the Clinic for Surgery, Zemun Clinical Hospital Center in Belgrade from January 2013 to September 2016. The study involved 60 patients with acceptable general operability and diagnostically verified malignant colorectal neoplasm. The patients were divided into two groups, each of 30 patients: the first group was composed of patients treated with open colorectal surgery; the second group were patients undergoing laparoscopically assisted colorectal surgery.

Three groups of factors were analyzed for all patients. The first group of factors was known preoperatively: age, sex, American Society of Anesthesiologists (ASA) score, preoperative values of hemoglobin, and localization. The second group of factors was known intraoperatively: we analyzed the potential differences of intraoperative complications, the duration of operations, blood and blood derivatives' compensation. The third group of factors were known postoperatively: complications, length of stay in intensive care, rate of peristaltic establishment and the time needed for unobstructed oral intake, number of hospitalization days, analgesic use, and verticalization time. The criteria for patient involvement in the study for both groups were as follows: patients with

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histopathologically diagnosed colorectal cancer, both sexes, age over 18 years, acceptable general operability, written consent for operative treatment.

Indications for surgical treatment were based on the guidelines issued by the Society of American Gastrointestinal and Endoscopic Surgeons [2]. Preoperatively, all the patients were prepared in terms of complete diagnostics for the diagnosis of colorectal malignancies. Colonoscopy was performed with biopsy and pathohistological analysis of the material, analysis of blood count and biochemistry, blood group, followed by supplementary diagnostic methods in the form of multi-slice computed tomography / magnetic resonance imaging of the abdomen and the pelvis, X-ray of the chest, due to preoperative determination of disease stage. Immediately the day before surgery, the patients discontinued oral administration, fluid reimbursement by infusion was administered in the form of solutions 0.9% NaCL, Ringer's lactate solution, Hartmann's solution, or 5% glucose solution. The patients were preoperatively administered an antibiotic in the form of second- and third-generation cephalosporins and metronidazole, as well as mandatory thromboembolic prophylaxis. The patients were operated on according to the regular operating program – electively in general endotracheal anesthesia. The following details of the surgical procedure were recorded in all the patients: duration of operation, amount of homologous blood transfused. Transfusion of blood products in the perioperative period was based on the hemoglobin level of 80 g/L or on an individual basis according to the clinical condition. All the patients were treated in accordance with a strictly controlled protocol with regard to analgesic administration, feeding, and postoperative care. Postoperative recovery of the bowel function was evaluated by first flatus and bowel movement. Postoperatively, the patients were transferred to the intensive care unit and then transferred to the Department of General Surgery as needed. Any anastomotic dehiscence with clinical and/or radiologic evidence was considered. The patients were discharged after meeting the following conditions: bowel movement and full recovery of both ambulation and oral food intake. Follow-up for infectious and noninfectious complications was carried out for 30 days after hospital discharge by weekly office visits.

The data required for this study were taken from the protocol of surgical treatment, patient medical history, therapy list of the patients, anesthesiology lists conducting surgical treatment and pathologist reports. All the data was grouped into two tables, which were subsequently used for statistical processing. The first table presented patients operated on by open surgical technique, while the second one showed patients operated on by a laparoscopically assisted surgical technique.

Descriptive and analytical statistical methods were used in this study. Of the descriptive ones used were the following: absolute and relative numbers (n, %), measures of central tendency (arithmetic mean, median), dispersion measures (standard deviation, interval of variation). Of the analytical statistical methods, the difference tests were used: parametric (t-test), non-parametric (χ^2 test, Fisher's exact probability test, Mann–Whitney U-test).

The choice of test depended on the data type and distribution. Parametric methods were used in situations where the distribution was normal, while non-parametric ones were used in situations where the distribution is not normal. The normality of the distribution was examined on the basis of descriptive parameters, normality distribution tests (Kolmogorov–Smirnov and Shapiro–Wilk test) and graphical methods (histogram, boxplot, QQ plot). The results are presented in tables and graphs. All the data were processed in IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA).

The study was done in accordance with the standards of the institutional committee on ethics.

RESULTS

The mean age of the patients was 67.5 years. The oldest patient in both groups was 86 years old, while the youngest one was 25 years old and underwent open surgery.

There was no statistically significant difference between the groups ($t = -0.697$; $p = 0.489$).

In the laparoscopic group of patients, 14 men and 16 women were represented. In the open patient group, 19 men and 11 women were represented. There was no statistically significant difference between the groups ($\chi^2 = 1.684$; $p = 0.194$). The third group of the ASA score patients was the highest in both groups, in as much as 68%. There was no statistically significant difference between the groups ($Z = -1.695$; $p = 0.090$). The mean hemoglobin in both groups was 126. There was no statistically significant difference between the groups ($t = 0.050$; $p = 0.960$). In the laparoscopic group, the largest percentage of malignancy was present in the region of sigmoid colon with 36.7%, while in the open surgery group the largest percentage of malignancy was in the area of the proximal third of the rectum with 43.3%. In the laparoscopic group the rectum was represented in 36%. All the patients had a diagnosed colorectal adenocarcinoma.

In the intraoperative group of factors, there were no significant intraoperative complications of the examined patient groups such as abundant abdominal bleeding and intraoperative lesions of the surrounding organs. The average operative time in the open group was 120 minutes, while in the laparoscopic group it was 156 minutes. The duration of the laparoscopic surgery is statistically significantly longer than open surgery group ($t = -4.783$; $p < 0.001$) (Table 1).

In the open group, blood transfusion was administered in nine patients. In the laparoscopic group, blood transfusion was administered in 10 patients. Intraoperatively, one dose of blood was administered in two patients in the laparoscopic group, while in the open group two patients were administered one dose of blood and one patient was administered two doses of blood. Postoperatively, in the laparoscopic group, four patients were administered one dose of blood each, and three patients two doses of blood. In the open group, two patients were administered a single dose of blood, three patients two doses of blood. There was

Table 1. Intraoperative factors in analyzed groups

Intraoperative factors	Open group (n = 30)	Laparoscopic group (n = 30)	p
Operative time (minutes)	120	156	< 0.001
Complications Organ injury Massive hemorrhage	/	/	/
Blood transfusion one dose two doses	2 1	2	0.781

Table 2. Postoperative complications in analyzed groups

Complications postoperative	Open group (n = 30)	Laparoscopic group (n = 30)	p
Lethal outcome	1	2	1.000
Anastomotic dehisces	3	2	
Intraabdominal hemorrhage	0	2	
Subcutaneous emphysema	0	1	
Wound infection	1	0	
Necrotizing fasciitis	0	1	
Clostridium difficult-colitis	1	0	
Urinary retention	2	1	

Table 3. Postoperative factors in analyzed groups

Postoperative factors	Open group (n = 30)	Laparoscopic group (n = 30)	p
ICU stay (days)	1.93	1.77	0.143
Peristalsis (days)	2.47	1.9	0.009
Oral intake (days)	3	2	< 0.001
Blood transfusion one dose two doses	4 3	2 3	0.781
Hospitalization (days)	9	5	< 0.001
Analgesics First degree Second degree	20 2.72	12 < 1	< 0.001 < 0.001
Verticalization (days)	2.9	1.9	0.001

no statistically significant difference ($\chi^2 = 0.077$; p = 0.781) (Table 2).

In the second group of factors, by analyzing postoperative complications in the two observed groups, there were no statistically significant differences between the groups ($\chi^2 = 0$; p = 1.000). There were two lethal outcomes in the laparoscopic group and one in the open group. Two patients in the laparoscopic group and three patients in the open group had anastomosis dehiscence ($\chi^2 = 0.218$; p = 1.000). In the laparoscopic group of patients, both dehiscences were treated by reoperation. One dehiscence in patients of the laparoscopic group was due to increased bleeding from stapler anastomosis. In the open group, two dehiscences were treated by reoperation, while one was treated conservatively. Postoperative intraabdominal hemorrhage was verified in the laparoscopic group of patients. In one case it was treated conservatively, while in another it was treated by reoperation. In the laparoscopic group, subcutaneous emphysema was verified in one patient and was spontaneously resolved. In the open group one patient had wound infection, there was one dehiscence of the wound, which were treated with suture. In the laparoscopic group of patients, a complication of necrotizing fasciitis was verified, which led to a lethal outcome. Clostridial intestinal infection in the form of pseudomembranous colitis was verified in one patient in the open group.

Two urinary retention rates were verified in the open and one in the laparoscopic group of patients (Table 3).

There was no statistically significant difference between the groups in length of stay in the intensive care unit (Z = -1.466; p = 0.143). There was, however, a statistically not significant difference: patients in the laparoscopic group averaged 1.77 days in the intensive care unit, while the patients of the open group averaged 1.93 days. A statistically significant (Z = -2.630; p = 0.009) earlier establishment of peristalsis was in a group of patients operated on laparoscopically. Peristalsis was established in 1.93 days on average in the laparoscopic group, while in the open group the average value was 2.47 days. There was a statistically significant difference in the rate of establishing undisturbed oral intake (Z = - 4.399; p < 0.001) – the average for the laparoscopic group was the second postoperative day, while for the open group it was the third postoperative day. In the laparoscopic group, an unhindered oral intake was established in seven patients on the first postoperative day, while in the open group of patients unhindered oral intake was not established before the second postoperative day. On average, the patients of both groups passed stool after the fourth postoperative day (Z = -0.811; p = 0.418). Postoperatively, the patients of the laparoscopic group were hospitalized for a statistically significantly (Z = -4.607; p < 0.001) shorter length of time (five days on average) in comparison to the patients of the open group (nine days on average).

The average number of doses of the first-degree analgesics administered to the patients of the laparoscopic group was12, while averagely 20 doses were administered to the patients undergoing open surgery. Less than one dose of the second-degree analgesics was administered in the laparoscopic group of patients, and an average of 2.72 analgesics were administered in the open group. Analgesics of the first (Z = -3.896; p < 0.001) and the second degree (Z = -2.303; p = 0.021) were statistically significantly less ordained in the laparoscopic group of patients than in the open group of patients. We found a statistically significant difference (Z = -3.341; p = 0.001) per patient verticalization day. Patients of the laparoscopic group were verticalized one day earlier on average than those of the open group. In the laparoscopic group, the patients were verticalized after 2.9 days on average, whereas in the open group this took place after 3.9 days on average. The earliest verticalization in the patients of the laparoscopic group was on the first postoperative day, while in the open group it was on the second postoperative day.

DISCUSSION

Following the introduction of laparoscopic cholecystectomy and its success in the treatment of gallbladder disease,

laparoscopic surgery began to be applied in other fields as well. Open colorectal cancer surgery has been considered the gold standard for surgical treatment of this disease for decades. With the advancement of technology and modern medicine, minimally invasive surgery is becoming the next step in the treatment of this disease. With the advent of laparoscopic procedures in the treatment of colorectal cancer, numerous papers on this topic have been published. They have showed an improvement in the quality of operative technique, and especially an advantage in the early postoperative recovery after this type of operative treatment [3, 4, 5]. At the very beginning, laparoscopically assisted colorectal surgery appeared promising and studies were done that confirmed that this type of surgery was less traumatic than open surgery. Leung et al. [6] in their study examined the systemic response of cytokines after laparoscopically assisted and classic resections of rectosigmoid carcinoma in 34 patients. Their results showed that trauma of the tissue, which is reflected in the cytokine response, is smaller after laparoscopic surgery. Theoretical advantage of colorectal cancer laparoscopic surgery over classic surgery is less painful operative wounds, and therefore less use of analgesics, earlier recovery of both bowel function and oral feeding, lower percentage infections of the surgical wounds, faster mobilization and shorter hospitalization of patients. Numerous studies have been done and some are ongoing, examining whether laparoscopic surgery has surpassed open surgery and whether it is able to fulfil adequately oncological radicality, which is of paramount importance [6, 7, 8].

In this study, the objective benefit of early postoperative recovery was evaluated in patients treated with laparoscopic surgery compared to those treated with classical colorectal surgery. We compared preoperative parameters between these two groups of patients, to show homogeneity in patient choice for both procedures. The mean age of the patients was 67.5 years. Regarding some studies that dealt with comparison of laparoscopic and open colorectal surgery, we can see benefits in patients over 70 years old treated with laparoscopic surgery, which shows a lower rate of postoperative mortality and morbidity. For elderly patients, of great importance is early mobilization, which is faster established in patients operated on by laparoscopic surgery [9, 10]. In several studies that analyzed risk factors for laparoscopic conversion colorectal surgery, one of the factors that proved statistically significant was obese male sex [11, 12]. The third group of ASA patients had the highest prevalence in both groups, in as much as 68%. A multicenter randomized Medical Research Council Conventional versus Laparoscopic-Assisted Surgery in Colorectal Cancer study concluded that the risk of conversion laparoscopic colorectal to open surgery rises in patients with ASA score of over 3 [13]. The ASA score is also an elevated independent predictor of postoperative mortality and morbidity [14]. Preoperative anemia is associated with poorer cancer response to therapy, poorer locoregional disease control, and overall shorter patient survival [14, 15]. The intraoperative and postoperative parameters that we compared showed a number of similarities with world studies done on this topic. In this study, there is a statistically significant difference in the operating time. The shorter operating time was in open surgery. We find similar data in a number of randomized studies, but conclusion of these studies is that laparoscopic colectomies are associated with improved outcomes compared with open operations that do not exceed an operative time of six hours. [16, 17]. The average operating time in the open group was 120 minutes, while it was 156 minutes in the laparoscopic group. We did not find significant difference between the duration of laparoscopic surgery when comparing our study to others. Nelson et al. [17] in their study involving 435 laparoscopic colorectal operations had an average operating time of 150 minutes. However, numerous studies indicate that continuous training of teams which are dealing with laparoscopic colorectal surgery, after multiple operations, reduces the duration of the operation [18, 19]. Hence, we can expect these two types of surgical treatment's duration to approximately equalize. In terms of reimbursement of blood and blood derivatives, we compared intraoperative and postoperative administrations of these products. The result of our research is that we did not obtain statistically significant difference in the ratio of the study groups. Our results coincide with a large meta-analysis by Japanese authors, who compared 12 papers – randomized studies – by comparing laparoscopic and classic colorectal surgery 1990–2011. This study included 4458 patients, which also showed no statistically significant difference in the reimbursement of blood transfusion [20]. It is considered that the advantage of laparoscopic surgery is the optical magnification of the operative fields, making the operational field, substrate, and surrounding structures more transparent. This fact should be in favor of lower intraoperative blood loss, and therefore reduced intraoperative and postoperative blood supply, which is confirmed by some papers [21]. Regarding intraoperative and postoperative complication, there was no statistical difference. This result show that laparoscopic colorectal surgery is as safe as open surgery. There is a difference, not statistically significant, in stay in the intensive care unit. Patients in the laparoscopic group spent 1.77 days in the intensive care unit, while patients in the open group spent 1.93 days there. A statistically significant difference was verified regarding the number of postoperative days hospitalized. We can agree with most studies that speak in favor of shorter postoperative hospitalization in patients operated on using laparoscopic surgery [22, 23]. The mean length of hospital stay in the laparoscopic group was five days. Similar results are shown in the study conducted by Lacy et al. [24], where average hospital stay was 5.2 days. Compared to some other randomized studies, our study differs from the study by Braga et al. [25], according to which the mean length of hospital stay was seven days for colon and 10 days for rectum, and from the COLOR study, where patients were averagely hospitalized 8.2 days [23]. By comparing the recovery of the bowel function and the recovery of oral food intake we found a statistically significant difference between the two groups, as some of the advantages of laparoscopic surgery are precisely in these two categories. Peristalsis was established in the laparoscopic group after 1.93 days, while in the open group it was established after 2.47 days. In a

study by Koh et al. [26], the recovery of the bowel function in the laparoscopic group is after 2.57 days. The COLOR II study has a slightly different results, with 1103 patients operated on laparoscopically, recovery of the bowel function was on the second day [27]. In our study, unobstructed oral intake was established on the second postoperative day on average in the laparoscopic group, while in the open group it was established on the third postoperative day. In the laparoscopic group, recovery of oral food intake on the first postoperative day was established in seven patients, while in the open group it was not established before the second postoperative day. The time of recovery of oral food intake in the study by Lacy et al. [24] was established on the second day, while it was almost on the third day in the COLOR study [23]. In the laparoscopic group, the patients were verticalized on average after 2.9 days, while in the open group this averaged 3.9 days. Most studies that compare laparoscopically assisted and classic colorectal cancer surgery support our results [23, 25, 26, 27]. As most other studies, we tried to express the degree of pain in patients through dose quantity of an administered analgesic. Pain management after colorectal surgery varies widely and predicts significant differences in patient-reported pain and clinical outcomes. Enhanced postoperative pain management requires dissemination of multimodal analgesia practices [28]. In our study, the analgesics we used were divided into the following two groups: first-degree analgesics – non-opioid analgesics (metamizole-sodium, ketorolac, diclofenac), and second-degree analgesics – opioid analgesics (tramadol). Studies showed a statistically significant difference in the administration of second-degree analgesics in terms of less administration of the analgesics in laparoscopic colorectal surgery.

CONCLUSION

Laparoscopically assisted surgery has an advantage over classical surgery colorectal cancer in regard to early postoperative recovery of the patient.

Conflict of interest: None declared.

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Значај лапароскопске хирургије за рани постоперативни ток болесника са колоректалним карциномом

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САЖЕТАК

Увод/Циљ Циљ наше студије био је поређење раног постоперативног опоравка код болесника оперисаних лапароскопски асистираном и отвореном методом код преоперативно хистопатолошки дијагностикованог колоректалног карцинома.

Методе У истраживању је учествовало 60 болесника са колоректалним карциномом, који су подељени у две групе од 30 болесника лечених отвореним или лапароскопским путем. Три групе фактора су прикупљене и анализиране за све болеснике. Прва група фактора били су старост, пол, *ASA* оцена, преоперативни хемоглобин, локализација тумора. Друга група параметара су интраоперативне компликације, трајање операције, надокнада крви и крвних деривата. Трећа група параметара били су постоперативне компликације, дужина боравка на интензивној нези, време отпочињања перисталтике и пероралног уноса, дужина хоспитализације, употреба аналгетика и време вертикализације.

Резултати Болесници који су били подвргнути лапароскопским операцијама показали су значајне предности у раном постоперативном опоравку у поређењу с онима који су били подвргнути отвореној операцији, у погледу броја постоперативних дана хоспитализације ($p < 0,001$), трајања операције ($p < 0,001$), дана успостављања перисталтике ($p = 0,009$) и дана успостављања несметаног оралног уноса ($p < 0,001$), време вертикације болесника ($p = 0,001$) и употребе аналгетика ($p < 0,001$).

Закључак На основу наших резултата може се закључити да лапароскопска хирургија карцинома колоректума има предност у односу на отворени оперативни приступ, што се тиче раног постоперативног опоравка болесника.

Кључне речи: лапароскопска колоректална хирургија; отворена колоректална хирургија; колоректални карцином

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Factors influencing early surgical outcomes of patients with acute aortic dissection type A

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SUMMARY

Introduction/Objective Even with the current treatment, mortality from aortic dissection remains high. The study aimed to evaluate the early postoperative outcome of patients with aortic dissection and identify which factors could influence it.

Methods The study included all consecutive patients who underwent surgery for acute aortic dissection type A from 2012 to 2017. We registered all parameters that could potentially impact the outcome (general data, medical history, clinical and cardiological diagnostic test findings, preoperative complications, type of cannulation and the operation performed, additional surgical procedures, operation duration, etc.). The patients were surgically treated according to the current protocols. The main outcome measures were complications and mortality during a one-month postoperative period. All data collected pre-, intra-, and postoperatively were compared and statistically analyzed.

Results The study included 246 patients, 57.54 ± 12.88 years old on average, and mostly of male sex (74%). Early postoperative mortality occurred in 17% of the patients. Preoperative chronic kidney insufficiency ($p = 0.005$) and cerebrovascular insult ($p = 0.047$) and tamponade ($p = 0.036$) were the major risk factors for postoperative complications and mortality. Long hypothermic cardiac arrest ($p = 0.001$), cross-clamp ($p = 0.017$) and cardiopulmonary bypass time ($p = 0.036$) increased postoperative complications. Postoperative complications started occurring after ≥ 33.5 minutes hypothermic cardiac arrest and ≥ 67.5 minutes cross-clamp time. Postoperative complications occurrence increased ($p = 0.034$), while performing anterograde cerebral perfusion decreased the frequency of lethal outcome ($p = 0.001$).

Conclusion The majority of patients surgically treated for acute aortic dissection had good postoperative outcome. However, numerous pre-, intra-, and postoperative factors can impact patient survival.

Keywords: acute aortic dissection type A; surgery; outcome; risk factors

INTRODUCTION

Aortic dissection (AD) occurs as a result of direct mechanical force acting on the aortic wall (hypertension, hypervolemia, loss of laminar blood flow through the aorta) and damage to the aortic wall (connective tissue disorders, atherosclerotic changes) [1]. The worldwide incidence of acute AD ranges 5–30 per million people [2, 3].

The survival and treatment outcomes of patients with acute AD type A have been continuously improving over the last decade [4]. However, even with the current treatment, due to potentially devastating complications, mortality from acute AD type A remains high. The most important and life-threatening complications of acute AD type A include lethal malperfusion syndrome, cardiac failure (myocardial infarction or cardiac tamponade) and stroke [5].

Numerous factors can impact the outcome of patients with surgically treated acute AD [1]. Some studies showed that the early survival of patients is affected by preoperative conditions like previous aortic valve replacement, migrating chest pain, limb ischemia, hypotension, shock, and cardiac tamponade. Additionally, long-term survival is influenced by preoperative renal function impairment, reduced left ventricular ejection fraction, and advanced age [5, 6].

The study goal was to investigate early postoperative outcome of patients with acute AD type A, treated surgically in our referral cardiac surgery center. Moreover, we aimed to identify which factors, in terms of the patients' preoperative characteristics, intraoperative surgical parameters, and postoperative complications could influence patient outcome.

METHODS

The study included all consecutive patients who underwent surgery for acute AD type A at the Cardiac Surgery Department, Dedinje Cardiovascular Institute in Belgrade, from January 1, 2012 to December 31, 2017. We considered all parameters that could potentially impact the acute AD type A patients' outcome.

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The main/primary tested effect was lethal outcome and the secondary indicator of the patients' condition were complications in the early postoperative period (30 days). Thus, we tested both which pre- and intraoperative parameters can cause postoperative complications, and how they all together impact patient survival. The study was approved by the institutional review board. All the patients signed informed consent for procedures and the study.

Preoperatively, general data (age, sex, smoking status) and medical history were taken. We registered whether investigated patients had chronic illnesses and preoperative complications such as arterial hypertension (pressure ≥ 140/90 mmHg), hyperlipoproteinemia (total cholesterol ≥ 4.5 mmol/L and low-density lipoprotein-cholesterol ≥ 2.5 mmol/L), cerebrovascular insult (CVI) previously or currently (ischemic stroke; transient ischemic attacks), periphery vascular disease (atherosclerosis except in the aorta), chronic kidney insufficiency (albuminuria > 30 mg/g; blood creatinine > 133 umol/L; glomerular filtration rate < 60 ml/min/1.73 m^2), heart tamponade, coronary illness (angina pectoris followed by acute coronary syndrome, i.e. myocardial infarction and unstable angina), and other minor cardiological symptoms/complications (fatigue, shortness of breath, heart palpitations, chest pain, cold extremities) [5].

Upon admission for surgery, the patients underwent a clinical and cardiology examination by transthoracic echocardiography and multislice computed tomography (MSCT) for visualization of the dissection localization and measurement of the diameters of the ascending, descending, and abdominal aorta. Only acute dissections of the type A (Stanford classification) were included in the study. Dissections were further divided into type I and II according to the DeBakey classification system. Finally, the EuroSCORE (www.euroscore.org) was determined for every patient.

The patients were surgically treated according to the current protocols for their condition (Bentall procedure; Interposition tube graft and resuspension of the aortic valve; Tirone David procedure; Hemiarch replacement and Arch replacement). The choice of operative technique for aortic reconstruction depended on the location of the primary endothelial tear [5]. In all cases, the open distal technique was performed in hypothermic cardiac arrest. Moreover, the primary entry resection was located and resected for all the patients. We registered the type of cannulation and the operation performed. It was also noted if anterograde cerebral perfusion, aortocoronary bypass and intervention on the mitral valve were performed. Moreover, we measured the deep hypothermic cardiac arrest time (DHCA) / hypothermic cardiac arrest time, cross-clamp time, and cardiopulmonary bypass (CPB) duration. The minimum DHCA temperature was 18°C.

Postoperatively, the patients were followed up for one month. During that period, all complications were registered, such as acute myocardial infarction, CVI, spinal cord injury, paralysis, kidney insufficiency, pneumonia, other minor complications (prolonged intensive care; the need for intubation; revision of hemostasis; uncomplicated urinary infection; uncomplicated wound infection) and/or lethal outcome.

Statistical analysis

All data collected pre-, intra-, and postoperatively were compared and statistically analyzed. The sample was portrayed by descriptive statistics (mean, standard deviation, frequency and percent). The Kruskal–Wallis χ^2 test was used to assess the differences in investigated parameters regarding postoperative outcomes. Receiver operating characteristics (ROC) analysis was used to find the cut-off operative time after which postoperative complications developed more often. Finally, we applied binary logistic regression to evaluate potential predictors of postoperative outcome in patients with acute AD type A. All investigated parameters were divided into two groups (preoperative and intra/postoperative) and in that manner used as dependent variables. We used IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA) and 0.05 was the significance level.

RESULTS

The study included 246 acute AD type A patients who were on average 57.54 ± 12.88 years old and were more often male (p = 0.001). There were no significant differences between the patients regarding their smoking status (p = 0.610). The most common operation performed in these patients was graft interposition. The longest CPB time was 3.4 hours. At least one preoperative chronic illness/complication was registered in 84.55% of patients (p = 0.001). Postoperative complications were also rather frequent (44.7%), but this finding was not significant (p = 0.097). Moreover, in the overall sample, the outcome was good for the majority of patients (80.9%; p = 0.001).

Data collected pre- and intraoperatively and the postoperative outcomes of the patients are presented in Tables 1, 2, and 3. The differences in general, pre-, and intraoperative data regarding postoperative outcomes are presented in Tables 4 and 5.

Out of all preoperative parameters only having chronic kidney insufficiency and tamponade on admission positively correlated with both early postoperative complications and lethal outcome. In addition, having CVI on admission was associated with postoperative complications, while more preoperative complications assessed together increased mortality in the early postoperative period of patients with acute AD type A.

Performing anterograde cerebral perfusion decreased the frequency of lethal outcome, while performing axillar cannulation and having early postoperative complications were associated with higher mortality. If duration of DHCA, cross-clamp time, and CPB time were longer, the patients had more early postoperative complications.

When we applied binary regression to investigate the association of the tested pre- and intraoperative parameters with lethal outcome in patients with acute AD type A, one

Table 1. Descriptive data of the investigated patients with acute aortic dissection

Parameters	Minimum	Maximum	Mean	SD
Patients' age	16	86	57.54	12.88
Ejection fraction	25	65	54.56	7.87
Ascending aorta diameter – millimeters	30	90	53.65	9.69
Descending aorta diameter – millimeters	0	55	32.45	7.81
Abdominal aorta diameter – millimeters	2	61	25.16	11.99
EuroSCORE	2	18.45	7.69	3.75
EuroSCORE 2	0.24	65.47	8.52	8.37
EuroSCORE Log	1.2	38.47	13.65	8.86
Deep hypothermic cardiac arrest time – minutes	13	52	31.56	11.86
Cross-clamp time minutes	53	192	64.43	7.52
Cardiopulmonary bypass duration – minutes	106	212	139.74	69.62

significant model was obtained. Early postoperative mortality could be predicted using intraoperative (B = -1.450; Wald = 71.495; OR = 0.235; R^2 Nagelkerke = 0.191; p = 0.001; classification = 80.53%), but not preoperative parameters (p = 0.096). Lethal outcome can be expected more often in patients with more postoperative complications and when patients spent more time on the cross-clamp (Table 6).

Table 2. General and preoperative acute aortic dissection patient data

Parameters		Frequency	%
Patients' sex	male	182	74
	female	64	26
Smoking status	not smokers	127	51.6
	smokers	119	48.4
Dissection type	one	213	86.6
	two	33	13.4
Marfan syndrome	no	227	92.3
	yes	19	7.7
Hypertension	no	75	30.5
	yes	171	69.5
Hyperlipoproteinemia	no	205	83.3
	yes	41	16.7
Cerebrovascular insult before operation	no	229	93.1
	yes	17	6.9
Periphery vascular disease	no	226	91.9
	yes	20	8.1
Chronic kidney insufficiency	no	237	96.3
	yes	9	3.7
Coronary illness before operation	no	213	86.6
	yes	33	13.4
Tamponade on admission	no	183	74.4
	yes	63	25.6
Cerebrovascular insult on admission	no	226	91.9
	yes	20	8.1
Type 1/2 aortic regurgitation	no	124	50.4
	yes	122	49.6
Type 3/4 aortic regurgitation	no	175	71.1
	yes	71	28.9
Other symptoms and complications	no	213	86.6
	yes	33	13.4
Had some preoperative complications	no	38	15.4
	yes	208	84.6

ROC analysis showed that DHCA time adequately explained 63.5% (p = 0.001), CPB time 57.6% (p = 0.052), and cross-clamp time envisaged 59.6% (p = 0.014) of postoperative complications' development (Figure 1). The cut-off for DHCA duration was 33.5 minutes (sensitivity = 65.3; specificity = 60.2), while it was 67.5 minutes for cross-clamp time (sensitivity = 60.2; specificity = 59.3).

DISCUSSION

AD of the aorta is an urgent surgical condition with high mortality due to the disease severity and the treatment complexity. Literature data show that as many as 50% of untreated patients with acute AD will die within the first 48 hours [2, 7]. Conversely, improvements in intraoperative management such as novel surgical techniques and post-surgical critical care have recently significantly improved the outcome for acute AD patients [4]. Nevertheless, even if patients are adequately surgically treated, in cases of severe acute AD type A, the mortality is around 25% [3, 8]. In our sample, early postoperative mortality was 16.9%, which is rather high, but similar to other populations

Table 3. Intraoperative parameters and postoperative complications

Parameters		Frequency	%
Cannulation type	apical	129	52.4
	femoral	41	16.7
	axillar	76	30.9
Cerebral perfusion	no	124	50.4
	antegrade	122	49.6
Operation type	graft interposition	132	53.7
	Bentall	55	22.4
	David	4	1.6
	hemiarch	4	1.6
	graft + hemiarch	38	15.4
	Bentall + hemiarch	13	5.3
Intervention on mitral valve	no	231	93.9
	yes	15	6.1
Aortocoronary bypass	no	204	82.9
	yes	42	17.1
Postoperative myocardial infarction	no	241	98
	yes	5	2
Cerebrovascular insult postoperatively	no	214	87
	yes	32	13
Postoperative paralysis	no	243	98.8
	yes	3	1.2
Postoperative kidney insufficiency	no	217	88.2
	yes	29	11.8
Other postoperative complications	no	162	65.9
	yes	84	34.1
Had early postoperative complications	no	136	55.3
	yes	110	44.7
Lethal outcome (30-day mortality)	no	199	80.9
	yes	47	19.1

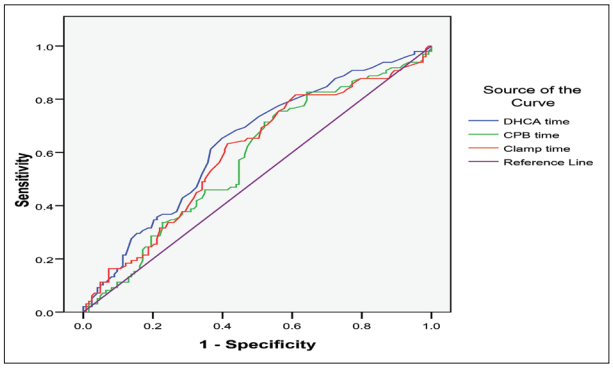

Figure 1. Receiver operating characteristics curve for postoperative complications based on operative times;
CPB – cardiopulmonary bypass; DHCA – deep hypothermic cardiac arrest time

from the literature. A potential cause for the high mortality could be the fact that a high percentage of patients were rather metabolically unstable preoperatively. Some data showed that higher admission creatinine value and C-reactive protein serum levels increase mortality of patients with acute AD [9].

The most common causes of death in acute AD type A (80%) are rupture of the aorta in pericardium with consequent tamponade (or tamponade without visible rupture of the aorta) and myocardial ischemia [10]. Tamponade occurs in 8–10% of cases and is one of the gravest complications and the worst prognostic signs [11, 12]. In our study, tamponade was registered in a somewhat higher percentage (16.4%), most likely due to the prolonged time from dissection onset to hospitalization. We confirmed that having tamponade on admission was associated with adverse outcome of patients with acute AD type A.

Aortic insufficiency is also one of the preoperative complications of acute AD type A correlated with poorer overall outcome [13]. In the literature, the incidence of aortic valvular insufficiency ranges 41–76% of cases [11]. In our sample, this percentage is slightly lower (31%) as we only investigated significant aortic insufficiency which required surgical treatment. We also did not prove that having significant aortic insufficiency preoperatively could increase adverse postoperative outcomes in patients with acute AD type A.

In some investigations, nearly 80% of patients with acute AD had ischemic lesions on MSCT. If neurological disorders are found preoperatively, adverse postoperative outcomes seem to occur more often [4, 14]. Mortality of acute AD type A patients with neurological complications reaches 50% in case of further intra- and postoperative complications. Still, patients with neurological deficits, with a favorable early postoperative course, usually recover fully [15]. In our study, preoperative neurological complications were not very frequent (around 7%), but having CVI on admission was associated with more postoperative complications.

Pre- and postoperative renal complications are risk factors for increased mortality in patients with acute AD type A [16]. The association between renal disease and worse cardiac surgery outcomes has multiple explanations [17].

Table 4. Differences in preoperative parameters regarding postoperative outcomes

Parameters	Early postoperative lethal outcome (yes/no)		Early postoperative complications (yes/no)	
	KW χ^2	p	KW χ^2	p
Dissection type	0.648	0.421	1.071	0.301
Marfan syndrome	0.146	0.702	1.441	0.230
Hypertension	0.882	0.348	0.927	0.336
Hyperlipoproteinemia	1.514	0.219	2.214	0.137
Cerebrovascular insult before	1.250	0.264	0.498	0.480
Periphery vascular disease	0.236	0.627	0.928	0.335
Chronic kidney insufficiency	7.998	0.005	4.114	0.043
Coronary illness before operation	0.021	0.885	0.008	0.927
Ejection fraction	1.783	0.182	0.301	0.583
Ascending aorta diameter mm	0.019	0.892	0.453	0.501
Descending aorta diameter mm	0.965	0.326	0.409	0.522
Abdominal aorta diameter mm	0.002	0.963	0.321	0.571
Tamponade on admission	4.487	0.036	2.921	0.087
Cerebrovascular insult on admission	0.487	0.485	3.906	0.047
Type 1/2 aortic regurgitation	1.428	0.232	0.827	0.363
Type 3/4 aortic regurgitation	0.041	0.840	0.045	0.833
Other preoperative complications/symptoms	1.198	0.274	0.218	0.640
Had some preoperative complications	4.607	0.034	0.123	0.725
EuroSCORE	0.824	0.364	3.143	0.076
EuroSCORE 2	0.156	0.693	0.842	0.359
EuroSCORE Log	0.053	0.818	0.515	0.473

Table 5. Differences in general and intraoperative data regarding postoperative outcomes

Parameters	Early postoperative lethal outcome (yes/no)		Early postoperative complications (yes/no)	
	KW χ^2	p	KW χ^2	p
Patients' age	1.248	0.264	0.001	0.971
Patients' sex	2.432	0.119	0.223	0.637
Smoking status	3.451	0.063	2.530	0.112
Cannulation type	12.981	0.001	1.714	0.190
Anterograde cerebral perfusion	11.977	0.001	0.392	0.531
Operation type	0.316	0.574	0.882	0.348
Intervention on mitral valve	0.588	0.443	0.143	0.705
Aortocoronary bypass	0.001	0.992	0.570	0.450
Deep hypothermic cardiac arrest time (minutes)	0.002	0.964	11.815	0.001
Cross-clamp time (minutes)	0.131	0.717	5.690	0.017
Cardiopulmonary bypass duration (minutes)	0.956	0.328	4.439	0.036
Had early postoperative complications	8.552	0.003	/	/

Patients with kidney disease may have more extensive coronary disease preoperatively, along with other comorbidities. In addition, impaired renal function can be a direct risk factor for intra- and postoperative complications, due to the need for greater fluid infusions or blood transfusions

Table 6. Significant model for prediction of acute aortic dissection operative outcome

	Parameters	B	Standard error	Wald	p	OR
Operative model	Cannulation type	-0.014	0.007	3.823	0.051	0.986
	Arrest Time	-0.007	0.009	0.608	0.435	0.993
	Anterograde perfusion	0.675	0.447	2.277	0.131	1.964
	CPB duration	0.005	0.003	2.599	0.107	1.005
	Cross clamp-time	0.541	0.239	5.111	**0.024**	1.718
	Operation type	0.095	0.107	0.799	0.371	1.100
	Aortocoronary bypass	0.060	0.531	0.013	0.910	1.062
	Intervention on MV	0.892	0.765	1.359	0.244	2.439
	Postop. complications	1.469	0.417	12.428	**0.001**	4.343
	Constant	-3.373	0.708	22.668	0.001	0.034

[16, 17, 18]. Having chronic kidney insufficiency on admission in our study was associated with both early postoperative complications and lethal outcome.

The incidence of acute AD type A correlates with age and it mostly occurs in the sixth decade of life. Men are at higher risk of developing acute AD type A than women. However, women tend to present at an older age, with more advanced dissection, and more complications, and therefore have a higher early mortality rate [1, 5, 6]. In our study, the majority of patients were also males around the age of 57 years, but neither sex nor age were significantly associated with early morbidity or mortality.

Hypertension is considered to be the most important risk factor for acute AD and is present in about 80% of acute AD type A patients [1, 5, 6]. Patients with hypertensive disorders lasting five or more years before the occurrence of acute AD type A have adverse outcomes more often than normotensive patients. Smoking is another risk factor for developing AD [12, 19]. However, we did not confirm that smoking impacted the early postoperative outcome of patients with acute AD type A.

Some investigations found that the rate of acute AD type A progressively rises along with the increase in aortic diameter. Aortic complications mostly start developing once the aortic diameter reaches 60 mm [2, 10]. In our sample, the average diameter of the ascending aorta was 53.65 mm, while that of the descending aorta was 32.45 mm. However, the aortic diameter was not associated with postoperative outcome.

Based on our results, no other preoperative patient characteristics and comorbidities were found affect the postoperative outcome of patients with acute AD type A. However, having more preoperative complications simultaneously did increase the rate of lethal outcome in the early postoperative period. One unexpected result was the fact that the EuroSCORE was not a significant predictor of outcome in our study. A possible explanation could be that our patients had few preoperative comorbidities that are assessed by EuroSCORE. Therefore, the average EuroSCORE was rather low in our study and consequently not sufficiently reliable for prediction. It seems that some other, not scored parameters and patient characteristics (perhaps biochemical and metabolic aspects) contributed to adverse outcome in our patients.

Treatment of acute AD type A continues to be challenging [2, 3]. Currently, different surgical techniques are being used for dissection treatment according to the indications, based on dissection type and patient condition. Adequate operative management remains a major concern for better outcome of acute AD type A patients [20, 21]. However, patients are completely different from one another, and numerous factors can impact the choice of technique and reflect surgical outcomes [22]. The results of our study show that, if appropriately chosen, the precise operation type was not a risk factor for postoperative morbidity and mortality.

Literature data show that aortic arch replacement was indicated in 12.2% of patients [12]. In our study, aortocoronary bypass, as a combined procedure with aortic reconstruction, was performed in 19.3% of patients. Studies indicate that early postoperative survival is equivalent when comparing antegrade and retrograde perfusion. Nevertheless, antegrade perfusion to the true lumen was associated with better long-term survival, while retrograde perfusion is a risk factor for late mortality [23, 24]. Our patients with antegrade perfusion also had better postoperative outcomes than those who underwent surgery without cerebral perfusion.

Currently, cannulation to establish CPB in patients with acute AD type A can be safely and efficiently performed through the femoral, subclavian, axillary artery, the ascending aorta, as well as through the left ventricular apex and the aortic valve [25, 26, 27]. Our results showed that axillar cannulation was associated with more frequent lethal outcome. A potential reason for this finding could be that patients who had axillar cannulation also had more preoperative chronic illnesses / complications, which might have influenced their overall outcome. Other authors also found that operative details differed significantly among the patients with different cannulation sites [21, 23]. Other cannulation sites were found to be safe for our patients.

As expected, emergency operations were confirmed to have a significantly higher risk for both postoperative mortality and morbidity than elective acute AD surgery [2]. However, despite different novel surgical modifications, significant improvement in early mortality was not observed [9]. Moreover, no significant link between the overall early mortality and the extent of the aortic repair has been proven in literature [20]. Contrary, it was found that perioperative complications were associated with the length of CPB, which again increased complications and mortality after surgery [10, 21]. In this study, it was found for the first time that if DHCA duration was ≥ 33.5 minutes and cross-clamp time was ≥ 67.5 minutes, postoperative complications were more likely.

Regression analyses performed in the literature suggest that the independent perioperative risk factors for adverse outcomes were prolonged cross-clamp and cerebral perfusion time [4]. Prolonged CPB, surgery time, and duration of deep hypothermia were the main intraoperative risk factors influencing surgical outcomes in patients who underwent aortic arch repair [20]. We found that performing the anterograde cerebral perfusion decreased lethal outcome.

In addition, shortening the duration of DHCA, CPB, and cross-clamp time could reduce early postoperative complications. The obtained results should be confirmed on a larger sample in the future for better reliability.

CONCLUSION

Our study shows that early 30-day mortality after surgery for acute AD type A remains high, affecting almost 17% of patients. Preoperative chronic kidney insufficiency, CVI, and tamponade are the major factors that could lead to more postoperative complications and potential adverse outcomes. Lethal outcome can be expected more often in patients with cumulative postoperative complications, and when patients spend more time on a cross-clamp. Moreover, we found that after 67.5 minutes of cross-clamp and 33.5 minutes of DHCA, postoperative complications occur more frequently.

Conflict of interest: None declared.

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Фактори који утичу на ране хируршке исходе болесника са акутном дисекцијом аорте типа А

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САЖЕТАК

Увод/Циљ Чак и уз тренутно лечење, смртност од дисекције аорте остаје висока.

Циљ студије био је да се евалуира рани постоперативни исход болесника са акутном дисекцијом аорте типа А и идентификује факторе који могу утицати на њега.

Методе Студија је обухватила све узастопне болеснике оперисане због акутне дисекције аорте типа А од 2012. до 2017. године. Регистровали смо све параметре који могу утицати на исход (општи подаци, историја болести, налази клиничких и кардиолошких дијагностичких тестова, преоперативне компликације, тип канулације и изведене операције, додатних хируршких поступака, трајање операције итд.). Болесници су хируршки лечени према важећим протоколима. Главне мере исхода биле су компликације и морталитет током једног месеца постоперативног периода. Сви подаци прикупљени преоперативно, интраоперативно и постоперативно упоређени су и статистички анализирани.

Резултати Студија је обухватила 246 болесника, просечне старости 57,54 ± 12,88 година и углавном мушког пола (74%). Рани постоперативни морталитет догодио се код 17% болесника. Преоперативна хронична инсуфицијенција бубрега ($p = 0,005$) и цереброваскуларни инсулт ($p = 0,047$) и тампонада ($p = 0,036$) били су главни фактори ризика за постоперативне компликације и морталитет. Дуго трајање хипотермичног срчаног застоја ($p = 0,001$), тоталне клеме ($p = 0,017$) и кардиопулмоналног бајпаса ($p = 0,036$) повећавали су постоперативне компликације. Постоперативне компликације почеле су да се јављају након ≥ 33,5 минута хипотермичног срчаног застоја и ≥ 67,5 минута времена тоталне клеме. Постојање више постоперативних компликација ($p = 0,034$) повећавало је, док је извођење антероградне церебралне перфузије смањивало учесталост смртног исхода ($p = 0,001$).

Закључак Већина болесника хируршки лечених због акутне дисекције аорте типа А имала је добар постоперативни исход. Међутим, бројни преоперативни, интраоперативни и постоперативни фактори могу утицати на преживљавање болесника.

Кључне речи: акутна дисекција аорте типа А; операција; исход; фактори ризика

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Audiological features in Serbian patients with hearing impairment identified with c.35delG in the *GJB2* gene

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SUMMARY

Introduction/Objective Hearing impairment is the most common sensorineural disorder with an incidence of 1/700–1000 newborns. Variants in the *GJB2* gene are the major cause of autosomal recessive nonsyndromic sensorineural hearing loss (ARNSHL). The degree of hearing impairment in patients with detected mutations in *GJB2* gene ranges from mild to profound.
The aim of this study was to determine possible genotype–phenotype association between audiometric characteristics and detected genotypes in ARNSHL patients from Serbia.
Methods Ninety-two patients with ARNSHL underwent genetic analysis with amplification-refractory mutation system polymerase chain reaction and sequencing of the *GJB2* gene. Audiological analyses were obtained in all patients using a combination of several methods to estimate the degree of hearing loss.
Results Audiological analysis performed in the 92 probands showed moderate to profound range of hearing loss. All identified pathogenic variants accounted for 42.39% of the mutant alleles (78/184 alleles), with the c.35delG mutation being the most frequent one (30.43%). Genotype–phenotype correlation in an isolated group of 37 patients bearing c.35delG in the homozygous, compound heterozygous, or heterozygous state. In this group the majority of patients (30/37, 81.08%) exhibited severe to profound hearing deficit.
Conclusion Association between genotype and the degree of hearing impairment in patients analyzed in this study demonstrated that patients with bi-allelic truncating mutations, i.e., c.35delG, associate with the more severe hearing loss when compared with those identified with only one affected allele. The various degrees of hearing impairment observed in heterozygous patients could be explained by the presence of an undetected second mutation or other modifier genes or environmental causes.
Keywords: hearing impairment; *GJB2* gene; c.35delG variant; audiological features

INTRODUCTION

Hearing loss is the most common sensorineural disorder in humans, with prevalence of one in 700–1000 children and 70% occurs as a nonsyndromic form [1–3]. Approximately half of these cases are genetic in origin [4]. The pattern of inheritance can be distinguished in autosomal recessive (loci named DFNB) (80%), autosomal dominant (DFNA) (17%), X-linked (DFNX) (2–3%), and mitochondrial forms (< 1%) [4].

The *GJB2* gene (the human gap junction β-2 gene - OMIM*121011) which encodes connexin 26 protein, was the first gene that was associated to nonsyndromic sensorineural hearing loss, and mutations in this gene are the most common cause of this disorder in many populations worldwide [1, 3]. More than 200 different pathogenic variants were identified in this gene [5, 6].

Because of the high number of identified variants, their frequency and distribution vary between populations, even within countries [1, 7, 8, 9]. Among these variants, c.35delG frameshift mutation is the most causative mutation of autosomal recessive sensorineural hearing impairment in Caucasians, with an incidence of 70% among the pathogenic alleles and a carrier rate of 1–3% [3, 4, 7–11].

The majority of mutations in connexin 26 are loss of function mutations, resulting in deprived permeability of channel or loss of gap junction and/or hemichannel function [12, 13]. The nonsyndromic hearing loss in patients with mutations in *GJB2* gene ranges from severe to profound and is generally nonprogressive [4, 7, 8].

The purpose of this study was to determine the possible genotype-phenotype association between the audiometric characteristics and the detected genotypes in a group of patients from Serbia with bilateral prelingual non syndromic sensorineural hearing loss.

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METHODS

Patients

Patients were initially clinically evaluated at the Unit for Audiology and Neuro-otology, which is a part of the Department of Otorhinolaryngology at the Dr. Vukan Čupić Mother and Child Health Care Institute of Serbia, during the period from April 2016 to June 2018. All the patients in this study had nonsyndromic bilateral sensorineural hearing impairment which corresponds to autosomal recessive pattern of inheritance (ARNSHL). The analyzed group consisted of 92 patients, 49 males and 43 females. The mean age at diagnosis of patients was 9.5 years, ranging from 3.5 months to 68 years. The protocol of this study was approved by the national Medical Ethics Committee (number 8/20, dated 29.06.2018.), and informed consent was obtained from all patients and/or parents/guardians.

Audiological analysis

Inclusion criteria for patients in the study was nonsyndromic bilateral sensorineural hearing impairment. In each patient, history of hearing loss, family history and physical examination were obtained to determine the age at onset of deafness and to exclude the possibility of environmental causes. Clinical evaluation was done to exclude syndromic hearing impairment by audiologist and pediatrician. Also, tympanometry was performed to exclude conductive hearing impairment. Based on all examinations on patients, it was concluded that patients have nonsyndromic bilateral sensorineural hearing impairment which corresponds to autosomal recessive pattern of inheritance. Children younger than four years (40 patients) underwent transient-evoked otoacoustic emission, brainstem-evoked response audiometry, and auditory steady-state response methods to estimate hearing loss. In addition to the mentioned methods, in patients older than four years (52 patients), the pure-tone audiometry with a diagnostic audiometer in a soundproof room was also done. According to the European protocol, the severity of hearing impairment in probands was classified as mild (20–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–95 dB), or profound (more than 95 dB) [4].

DNA analysis

Peripheral blood samples of all patients and their relatives were collected for genetic analysis. DNA analyses were initially performed at the Laboratory of Medical Genetics of the Mother and Child Health Care Institute of Serbia. Sequencing of the coding exon 2 of the *GJB2* gene was performed at the Department of Molecular Genetics, Function and Therapy at the Cyprus Institute of Neurology and Genetics. Genomic DNA was extracted from the peripheral blood samples with ethylenediamine tetraacetic acid using GenJet™ Genomic DNA Purification Kit (ThermoFisher Scientific Inc, Waltham, MA, USA) as instructed by the manufacturer.

Amplification-refractory mutation system polymerase chain reaction for direct detection of c.35delG variant in the GJB2 gene

Direct detection of c.35delG mutation in the *GJB2* gene was carried out in all the patients, using the amplification-refractory mutation system polymerase chain reaction (ARMS-PCR) method, according to modified original method [14, 15]. In two separate PCR reactions, three primers, one common reverse, one normal forward, and one mutant forward were used (size of amplicon at 202 bp). As an internal control of both reactions, two additional primers for amelogenin were used (size at 360 bp) [16]. DNA of mutant homozygote, heterozygote, and wild type samples as controls were run in parallel for each PCR set. Products were separated by horizontal electrophoresis on 2% agarose gel and visualized by staining with ethidium bromide and exposure to ultraviolet light.

Direct sequencing of exon 2 in the GJB2 gene

All the patients found negative or identified in heterozygosity for the c.35delG were further screened by direct sequencing of exon 2 of the *GJB2* gene as previously described [15]. Direct sequencing of the entire coding sequence of the *GJB2* gene was performed using BigDye terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA), according to manufacturer's procedure, on an ABI PRISM® 3130XL Genetic Analyzer (Applied Biosystems). Internal primers were also used for the complete cover-up of the coding region of the *GJB2* gene, as described by Neocleous et al. [15].

After receiving results from genetic testing, all families at risk were offered genetic counselling.

RESULTS

Audiological analysis

As described previously, all patients included in this study had ARNSHL which corresponds to autosomal recessive pattern of inheritance. The tympanometry excluded conductive type of deafness in probands. Family history and physical examination excluded the possibility of environmental causes. Audiological analysis performed in the 92 probands demonstrated moderate to profound range of hearing loss (ranging from 41–115 dB) (Figure 1). According to the European Molecular Genetics Quality Network Best Practice Guidelines, patients were classified as having moderate, moderately severe, severe, and profound range of hearing impairment (Table 1) [4]. Mostly they had severe to profound hearing impairment (63/92 patients, 68.48%), while only 6 (6/92, 6.52%) had moderately severe range.

DNA analysis

Ninety-two Serbian patients with autosomal recessive nonsyndromic bilateral sensorineural hearing impairment

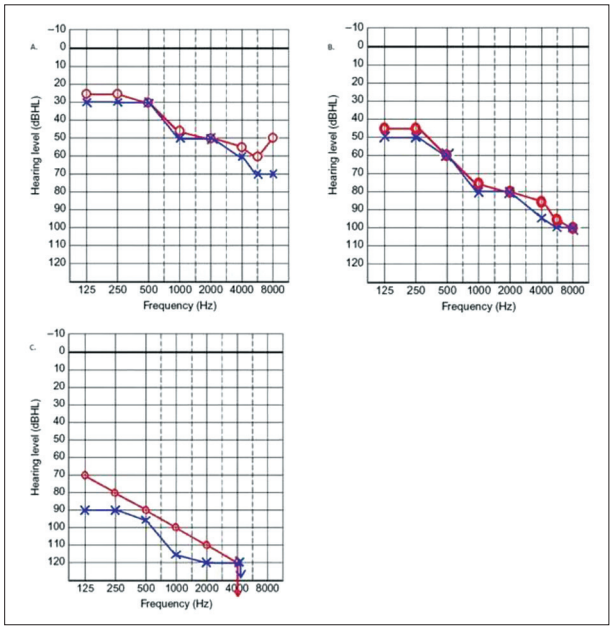

Figure 1. Audiograms in patients with A – moderate, B – severe, C – profound degree of hearing impairment;
o – right ear; × – left ear

Table 1. Results of audiological analysis in a group of 92 Serbian patients with nonsyndromic sensorineural hearing loss

Range of hearing impairment	n (%)
Moderate	23 (25%)
Moderately severe	6 (6.52%)
Severe	29 (31.52%)
Profound	34 (36.96%)
Total	92 (100%)

were initially screened for the presence of c.35delG mutation in the *GJB2* gene. Using the ARMS-PCR method, the c.35delG mutation was detected in 37/92 (40.22%) probands. In this group, c.35delG was nearly equally presented in homozygous and heterozygote state (19/37 and in 18/37 tested patients, respectively) (Figure 2) (Table 2). Further screening of the coding exon 2 was performed in patients found negative or identified in heterozygosity for the c.35delG mutation. In four probands sequencing analysis revealed a second pathogenic variant, while 14 patients had c.35delG in the heterozygous state only (Table 2). In total, all identified pathogenic variants accounted for 42.39% of the mutant alleles (78/184 alleles), while the rest remained uncharacterized. The most frequent mutation was c.35delG mutation and appeared in 56/184 alleles

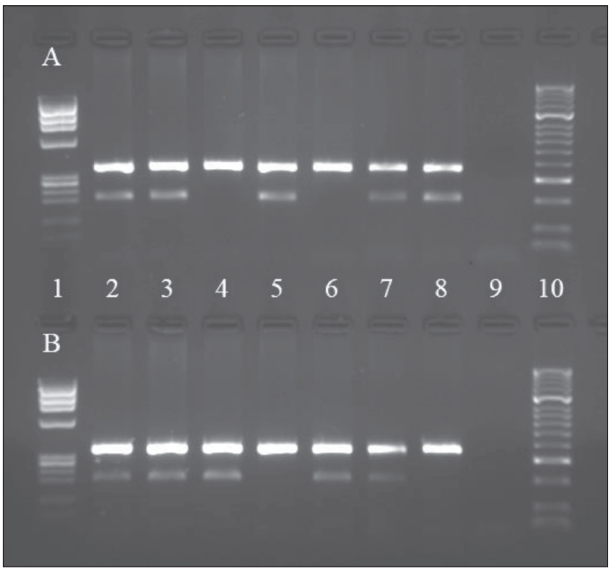

Figure 2. Polymerase chain reaction (PCR) amplification-refractory mutation system products for direct detection c.35delG mutation (202 bp) with internal control (amelogenin, 360 bp), 2% agarose gel, 85V, 1h; A: PCR reaction for detecting normal allele; B: PCR reaction for detecting mutant allele; lane1: GeneRuler 1kb DNA ladder; lanes 2 and 3: samples heterozygous carriers; lane 4: homozygote sample; lane 5: sample wild-type; lane 6: homozygote for the c.35delG control–sample; lane 7: heterozygous carrier control sample; lane 8: wild type control sample; lane 9: blanc; lane 10: ΦX174 DNA-Hae III Digest DNA ladder

(30.43%). To confirm homozygosity and or compound heterozygosity identified in the probands, genetic testing was also done in parents and close relatives (140 relatives in total). Genetic counseling was recommended to all families at risk, considering possibilities of carrier testing and prenatal screening.

Audiologic features in carriers of c.35delG mutation

Thirty-seven of the examined patients were homozygous or heterozygotes for the c.35delG mutation. The majority of these patients were homozygous for the c.35delG mutation (19/37) and had profound degree of hearing impairment (11/19, 57.89%). Four patients (4/37) were found to be compound heterozygotes for the c.35delG mutation and a second pathogenic variant in the *GJB2* gene: c.[35delG];[c.313_326del] in three patients, c.[35delG];[c.71G>A] detected in one patient. All of these patients exhibited profound hearing impairment. The identified patients with only one c.35delG mutation on the one allele and an expected unidentified second mutation

Table 2. Genotype frequencies for the 35delG mutation and association between genotype and degree of hearing impairment in a group of 37 Serbian patients

Genotype	n of probands (%)	Percentage in total cohort of 92 patients	Range of hearing impairment (n of probands)			
			Moderate	Moderately severe	Severe	Profound
c.[35delG];[c.35delG]	19 (51.35%)	20.65	0	2	6	11
c.[35delG];[=]*	14 (37.84%)	15.22	5	0	5	4
c.[35delG];[+]**	4 (10.81%)	4.35	0	0	0	4
Total	37 (100%)	40.22	5	2	11	19

=* – unidentified second pathogenic variant;
+** – identified second pathogenic variant

Table 3. Frequencies of the c.35delG mutation homozygote and compound heterozygote carriers per region

Population	Study	n of probands	c.35delG homozygote carriers (% in total cohort)	c.35delG compound heterozygote carriers (% in total cohort)
Romania	Resmerita et al. [18]	291	58.76	14.4
Hungary	Battellino et al. [24]	194	35.6	12.6
Greece	Battellino et al. [24]	210	30	6.2
Czechia	Battellino et al. [24]	156	28.8	5.1
Croatia	Battellino et al. [24]	63	25.4	7.9
FYR Macedonia	Sukarova Stefanovska et al. [23]	130	25.38	3.85
Slovenia	Battellino et al. [24]	218	21.1	10.5
Serbia	Present study	92	20.65	4.35

(14/37) exhibited moderate to profound hearing loss (Table 2). Finally, our results demonstrated that all carrier patients bearing c.35delG mutation had predominantly severe to profound degree of hearing impairment (30/37, 81.08%).

DISCUSSION

The degree of hearing loss associated with two allele variants in the *GJB2* gene is variable, ranging from mild to profound [8]. A large multicenter genotype–phenotype correlation study by Snoeckx et al. [7] demonstrated that mutations in the *GJB2* gene inactivate the protein product (connexin 26) and cause a more severe phenotype compared to those that do not fully inactivate connexin 26 [8]. Interestingly, this same correlation study also identified variability even among patients with the same genotype. Another study by Cryns et al. [17] also suggested that the severity of hearing impairment is mostly determined by a specific combination of mutations in the *GJB2* gene and that there is no difference in phenotypes in patients with the same or similar genotypes, with different geographical and/or ethnic origin.

Nowadays, c.35delG mutation is widely known as the most prevalent mutation in Caucasians either in the homozygous state or compound heterozygote state in *GJB2* or *GJB6* genes [4, 11]. c.35delG is a truncating, frameshift mutation that leads to premature protein synthesis termination at the 12th amino acid [18]. In the present study, the total frequency of the c.35delG mutant allele was estimated at 30.43% (56/184 alleles) and was identified in 37 patients either in homozygote or heterozygote state (40.22%) (Table 2). The recovered numbers concerning homozygosity and/or heterozygosity of the c35delG are quite similar to the ones previously reported in neighboring populations [10, 18–24] (Table 3).

Nineteen homozygous patients for c.35delG were placed in group 1, since they exhibited severe to profound bilateral sensorineural hearing impairment (Table 2). Among the 19 patients of group 1, 11 exhibited a profound degree of hearing loss with over 100 dB. Only two patients exhibited moderately severe degree of hearing impairment with ~60 dB. Our results are in agreement with many published studies worldwide [18, 19, 25, 26]. In general, the majority of homozygotes for the c.35delG mutation exhibit severe to profound hearing impairment, while few cases develop moderate to even mild hearing impairment [2, 3, 7, 8].

Previously published data showed that there are no significant differences between the audiological parameters in probands who are homozygotes for the c.35delG and compound heterozygous with c.35delG on the one allele [17]. The obtained results of the present study are also in agreement with these findings. In group 2 of the present study there were four patients (4.35% of the total cohort) with the compound heterozygous genotype, all of whom demonstrated profound hearing loss (Table 2). Similar results were found in compound heterozygotes from Romanian and Croatian populations identified in 4.8% and 7.9% of the total cohort, respectively, who mostly exhibited profound degree of hearing impairment [18, 19].

In group 3 of the present study, 14 patients (15.22%) were identified with only one pathogenic mutation and a possible second pathogenic variant remained unidentified due to limitation of the Sanger sequencing methodology. The identification of a possible second pathogenic mutation could be the subject of further analyses using the high-throughput next-generation sequencing technology. The severity of hearing impairment in group 3 ranged from moderate to profound. Therefore, phenotypic variability in group 3 could be further and more precisely explained by the effect of second unidentified disease-causing mutation. The possibility of this phenotypic variability being influenced by modifier gene(s) and/or environmental factors is likable. Previously published data from functional studies suggest that such contribution as a result of modifier genes is considered to be less important [2, 3, 17].

CONCLUSION

The association between genotype and the degree of hearing impairment in patients analyzed in the present study demonstrate that patients with the bi-allelic c.35delG associate with a more severe hearing loss when compared to the ones identified with only one affected allele. Additionally, to some extent, and in agreement with previous studies, the genotype-phenotype correlation in many cases remains controversial and definitely needs further genetic investigation, possibly with the emergence of the next-generation sequencing technology for the identification of other causing genes.

Conflict of interest: None declared.

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Аудиолошке карактеристике код болесника из Србије са оштећењем слуха са идентификованом мутацијом *c.35delG* гена *GJB2*

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САЖЕТАК

Увод/Циљ Оштећење слуха представља најчешће сензоринеурално обољење и јавља се са учесталошћу од 1/700–1000 новорођенчади. Варијанте у гену *GJB2*, који кодира протеин конексин 26, најчешћи су узрок аутозомно-рецесивног несиндромског оштећења слуха. Код пацијената са детектованим мутацијама у гену *GJB2* степен оштећења слуха варира од умереног до дубоког.

Циљ овог рада је био утврђивање могућих генотип–фенотип асоцијација између идентификованих генотипова и аудиометријских карактеристика код пацијената из Србије.

Методе Генетичке анализе су спроведене код 92 пацијента помоћу методе *ARMS-PCR* и секвенцирања кодирајућег региона гена *GJB2*. Аудиолошке анализе за процену степена оштећења слуха су урађене код свих пацијената комбинацијом више метода.

Резултати Резултати аудиолошких анализа код 92 пацијента показали су умерени до дубоки степен оштећења слуха. У 42,39% алела идентификоване су патогене варијанте (78/184 алела), а најчешће детектована је *c.*35*delG* варијанта са учесталошћу од 30,43%. Асоцијације генотип–фенотип су испитиване на изолованој групи пацијената (37), који су хомозиготни, сложени хетерозиготни или хетерозиготни носиоци *c.*35*delG* варијанте. У овој групи пацијената најчешћи степен оштећења слуха је тежак до дубок (30/37, 81,08%).

Закључак Код пацијената анализираних у овом раду асоцијација између генотипа и оштећења слуха показује да се код пацијената са биалелским мутацијама (међу којима је *c.*35*delG*) чешће јавља тежи облик оштећења него код пацијената са мутацијом на једном алелу. Већа варирања у степену оштећења слуха код хетерозиготних болесника могу бити објашњена ефектом друге, неидентификоване мутације, генских модификатора или ефектом средине.

Кључне речи: оштећење слуха; ген *GJB2*; варијанта *c.*35*delG*; аудиолошке карактеристике

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Videolaryngostroboscopy in early vocal fold carcinoma diagnosis

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SUMMARY

Introduction/Objective Vocal folds are the most common primary site of laryngeal carcinoma. Advancement in diagnostic and therapeutic modalities have provided better prognosis for patients with early glottic carcinoma. We aimed to report the role of videolaryngostroboscopy (VLS) in early diagnosis of vocal fold carcinoma.

Methods Prospective controlled study included 300 dysphonic patients admitted to the tertiary medical center for microlaryngoscopy with biopsy. All patients underwent stroboscopic examination prior to biopsy. VLS findings were classified according to Hirano into four stages, with an adynamic vocal fold segment and absence of vocal fold vibration, suspected for vocal fold carcinoma at stage IV. Histopathological findings have been graded according to Ljubljana classification into simple hyperplasia, abnormal hyperplasia, atypical hyperplasia, and carcinoma *in situ*.

Results Analysis of VLS findings showed that 41.67% of patients (n = 125/300) had asymmetrical and irregular vocal fold vibration with a mucosal wave reduction (VLS stage III), while an adynamic vocal fold segment and absence of vocal fold vibration (VLS stage IV), suspected for vocal fold carcinoma, was noticed in 17.33% of patients (n = 52/300). Histopathology report showed that vocal fold carcinoma was verified in 5.6% of patients in VLS stage III (n = 7/125), while VLS stage IV carcinoma was detected in 26.92% of patients (n = 14/52). Adynamic segment or entire nonvibrating vocal fold finding predicts early glottic carcinoma with a sensitivity of 66.77%, specificity of 86.4%, and moderate diagnostic accuracy (AUC = 0.844).

Conclusion VLS plays an important role as a timely indicator for microlaryngoscopy with biopsy in diagnosis of vocal fold carcinoma.

Keywords: videolaryngoscopy; vocal fold carcinoma; microlaryngoscopy

INTRODUCTION

Laryngeal cancer accounts for 30–40% of malignant head and neck tumors and for 1–2.5% of all malignancies. Squamous cell carcinoma adds up to 95–98% of laryngeal cancers. The occurrence of laryngeal carcinoma is more common in male patients between the fifth and seventh decade of life. Laryngeal carcinomas are one of several oncological diseases in which the five-year survival rate has decreased from 66% to 63% over the last 40 years, although the total incidence is declining [1, 2, 3]. In Serbia, laryngeal carcinomas are one of the most common malignancies, and, according to 2017 data, they rank sixth in terms of occurrence [4].

The first clinically manifested symptom of vocal fold carcinoma is dysphonia. According to American Academy of Otorhinolaryngology and Head and Neck Surgery guidelines, dysphonia diagnostics includes anamnesis, clinical examination focusing on laryngeal motility and visible pathological changes of the vocal folds, videolaryngostroboscopy (VLS) and microlaryngoscopy with biopsy as gold diagnostic standard. The occurrence of vocal fold carcinoma is associated with smoking, gastroesophageal reflux disease, HPV virus, with alcohol being less involved in the development of vocal fold cancer than in other localizations of laryngeal tumors. VLS provides insight in anatomical structures and functional changes of the vocal folds (appearance and vibratory patterns), without complications. Though it is invasive procedure, microlaryngoscopy is necessary complementary method to VLS, and without it histological confirmation of the lesion would be impossible [5, 6, 7].

The aim of this study is to distinguish the presence of early glottic carcinoma in patients with or without dysplastic lesions of vocal folds, using certain clinical characteristics observed during VLS.

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METHODS

This prospective study with 300 dysphonic patients was conducted over a four-year period in the Clinic of Otorhinolaryngology, Kragujevac Clinical Center, Serbia. Ethics Committee of the Kragujevac Clinical Center approved the study. Patients with functional voice disorders, benign tumors, pseudotumors, as well as patients with verified vocal fold carcinoma were excluded from the study. All the patients underwent detailed anamnesis, otorhinolaryngological and phoniatric examination, VLS, and microlaryngoscopy with biopsy. All the subjects gave their informed consent for participation in the study and ethics guidelines of the Declaration of Helsinki were followed during the study.

VLS was performed using a rigid Karl Storz stroboscope (Karl Storz SE & Co. KG, Tuttlingen, Germany). One doctor performed and evaluated all stroboscopic examinations. Following parameters were analyzed: glottic occlusion, amplitude and regularity of vocal fold vibration and presence of mucosal wave. VLS findings were classified according to Hirano into the following four stages: 1. Insufficient glottic occlusion with symmetrical, regular vocal fold vibration with regular amplitude and presence of mucosal wave; 2. Insufficient glottic occlusion with symmetrical and regular vocal fold vibration with a reduced amplitude, and slightly reduced mucosal wave; 3. Insufficient glottic occlusion with asymmetrical and irregular vocal fold vibration with a mucosal wave reduction; 4. Adynamic vocal fold segment and absence of vocal fold vibration, suspected for vocal fold carcinoma [5, 8].

Microlaryngoscopy with biopsy was performed in general anesthesia with SOM 62 microscope (Karl Kaps GmbH & Co. KG, Asslar, Germany) and Karl Storz laryngoscope (Karl Storz SE & Co. KG). All sections of the obtained samples had been embedded in paraffin wax, cut at 3–5 mm thickness from at least two parts of the paraffin block and stained with hematoxylin and eosin for histopathological (HP) analysis. HP findings have been graded according to Ljubljana classification into simple hyperplasia (benign spinous layer augmentation), abnormal hyperplasia (benign basal and parabasal layer augmentation), atypical hyperplasia (risky for malignancy) and carcinoma *in situ*. [9, 10].

The data were analyzed using statistical package IBM SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY, USA). The normality of distribution was tested by the Kolmogorov–Smirnov test. The statistical association was evaluated using the χ^2 test. Receiver operating characteristic (ROC) curve is used for the assessment of VLS diagnostic values. Diagnostic value is tested using clinical variables of interest by the method of logistic regression. Diagnostic value of VLS was evaluated through determination of sensitivity, specificity, and diagnostic accuracy. The results were considered significantly different when $p < 0.05$.

RESULTS

Our study included 220 male (73.33%) and 80 female patients (26.67%), with an average age of 50.59 years. The smokers accounted for 88.67% (n = 266/300) of patients, with a 23.970 ± 12.651 mean smoking years history, while 11.33% (n = 34/300) of patients were non-smokers.

Analysis of VLS findings according to the Hirano classification showed that all patients had insufficient glottic occlusion. In 27.33% of patients (n = 82/300) we noticed symmetrical, regular vocal fold vibration with regular amplitude and presence of mucosal wave (VLS stage I), 13.67% of patients (n = 41/300) had symmetrical and regular vocal fold vibrations with a reduced amplitude, and slightly reduced mucosal wave (VLS stage II), while 41.67% of patients (n = 125/300) had asymmetrical and irregular vocal fold vibrations with a mucosal wave reduction (VLS stage III). Adynamic vocal fold segment and the absence of vocal fold vibration (VLS stage IV), suspected for vocal fold carcinoma, was noticed in 17.33% of the patients (n = 52/300) (Table 1).

Table 1. Number of patients with specific epithelial hyperplastic laryngeal lesions distributed by videolaryngostroboscopy (VLS) stages

Epithelial hyperplastic laryngeal lesions	VLS stages				Total
	Stage I	Stage II	Stage III	Stage IV	
Simple hyperplasia	73	17	33	3	**126 (42%)**
Abnormal hyperplasia	5	17	46	18	**86 (28.67%)**
Atypical hyperplasia	4	7	39	17	**67 (22.33%)**
Carcinoma	0	0	7	14	**21 (7%)**
Total (%)	82 (27.33)	41 (13.67)	125 (41.67)	52 (17.33)	300

All the patients underwent microlaryngoscopy with vocal fold biopsy. HP report showed that simple hyperplasia was present in 42% of the patients (n = 126/300), abnormal hyperplasia in 28.67% of the patients (n = 86/300), 22.33% of the patients (n = 67/300) had atypical hyperplasia, while 7% of the patients (n = 21/300) were diagnosed with laryngeal carcinoma (Table 1).

In VLS stage I, most common HP finding was simple hyperplasia. Carcinoma was not detected in VLS stages I and II. In VLS stage III carcinoma was verified in 5.6% of the patients (n = 7/125), while VLS stage IV carcinoma was detected in 26.92% of patients (n = 14/52) (Figure 1a, b). HP reports indicating carcinoma showed that 52.38% of the patients (n = 11/21) had carcinoma *in situ*, while 47.62% of the patients (n = 10/21) had invasive laryngeal carcinoma. Our results showed that there were significantly more patients with carcinoma in VLS stage IV than in other VLS stages (Figure 1b).

To examine diagnostic significance of VLS in carcinoma diagnostics we used ROC curve for determining the procedure sensitivity and specificity, univariate and bivariate logistic regression. Adynamic segment finding had 0.667 sensitivity and 0.864 specificity in detecting vocal fold carcinoma [area under the curve (AUC) = 0.844, 95% confidence interval (CI) 0.772–0.916)]. Odds ratio for

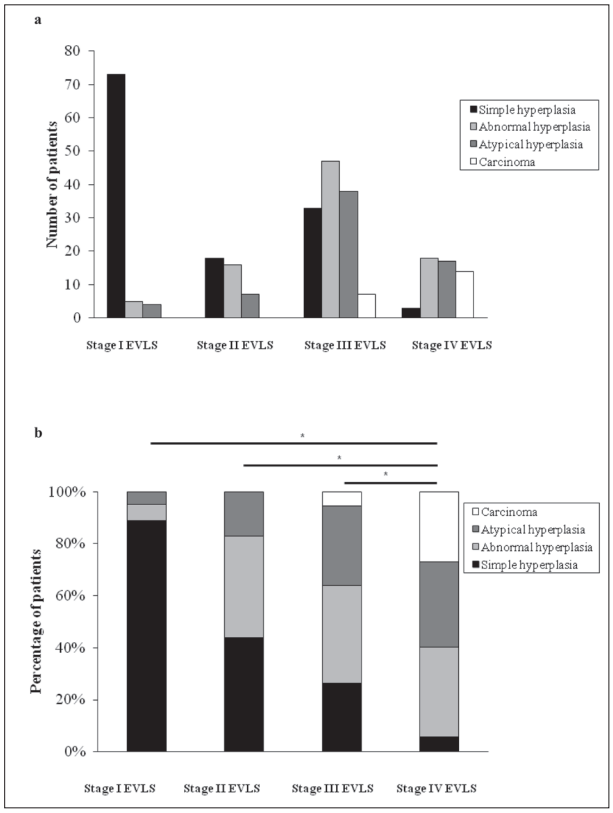

Figure 1. Distribution of epithelial laryngeal lesions according to endovideolaryngostroboscopy (EVLS) findings

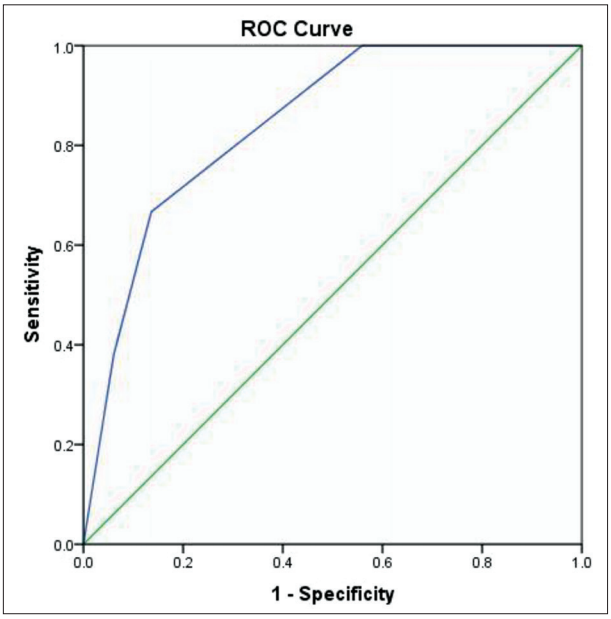

Figure 2. The area under receiver operating characteristic (ROC) curve for the sensitivity and specificity of videolaryngostroboscopy in vocal fold carcinoma diagnosis

VLS findings of 3.44 (95% CI 2.14–5.51) indicates that for each gradation of findings, from VLS stage I to stage IV, the chance for carcinoma detection after biopsy is increased. Our results imply a cut-off point for early diagnostics of glottic carcinoma with a sensitivity of 66.77%, specificity of 86.4%, and moderate diagnostic accuracy (AUC = 0.844) for early detection of glottic carcinoma. According to our results, VLS predicts most precisely early glottic carcinoma when adynamic segment or when entire nonvibrating vocal fold is found (Figure 2).

DISCUSSION

There is a great interest in the assessment of the VLS role in diagnosis of vocal fold carcinoma and benign pathology [9, 10]. VLS is considered to be an objective method with a subjective interpretation of the experienced endoscopists [5, 11]. In our study, VLS was used as a diagnostic procedure, while biopsy with HP evaluation as a gold diagnostic standard was a comparative method used to confirm stroboscopic prediction.

Analyzing the VLS and HP reports on 112 subjects, Jotic et al. [5] found that the adynamic segment was present in 15.1% of patients with mild, 38.5% of patients with moderate, and in 54.5% of patients with severe dysplasia (carcinoma *in situ*). In a prospective clinical trial of 66 patients, VLS findings were compared with histopathological verification of glottic carcinoma. Asymmetrical and irregular vocal fold vibrations with absent mucosal wave or absent vibrations of one part or the entire vocal fold were histopathologically confirmed as cancer in 85% of the patients. There were seven HP reports positive for carcinoma (87.5%) and one negative (12.5%) in the group of patients with absent mucosal wave. In the group of patients with absent vocal fold vibrations, there were 49 positive (84.48%) and nine negative (15.52%) HP reports. The authors concluded that in the cases of hoarseness present more than 14 days, VLS is the method of choice in assessing the need for microlaryngoscopy and HP verification [12]. El-Demerdash et al. [13] found that the sensitivity and specificity of VLS in predicting the invasive nature of vocal fold lesions based on the absence or reduction of the mucosal wave was 96.8% and 92.8%, respectively. Caffier et al. [14] found absence of vocal fold vibrations and adynamic segment (VLS stage IV) in 17/34 patients, while in 16 patients, malignancy was histopathologically confirmed. In addition, Gugatschka et al. [15] showed that the combination of exfoliative cytology and VLS allows for the detection of glottic cancer with a sensitivity of 97%. In compliance with the available data, our results showed that 26.92% of the patients (14/52) with adynamic segment and absent vocal fold vibration had histopathologically confirmed vocal fold carcinoma. In addition, in 66.67% of the patients (14/21) with vocal fold carcinoma adynamic segment and absent vocal fold vibration were detected (Figure 1). Adynamic segment or absent vocal fold vibrations predict early glottic carcinoma with a sensitivity of 66.77%, specificity of 86.4%, and moderate diagnostic accuracy, AUC = 0.844 (Figure 2).

Gamboa et al. [16] used the WHO histopathology classification, which is compatible with the Ljubljana classification that we used in our study. Absent mucosal wave as a suspected VLS finding was observed in 15 patients. Severe dysplasia was histopathologically confirmed in 13.4% of

the patients (n = 2/15), and planocellular carcinoma in 46.7% (n = 7/15). Histopathology did not show cell atypia in 26.7% of the patients (n = 4/15), while remaining 13.4% (n = 2/15) showed mild dysplasia.

VLS findings that are characteristic of chronic laryngitis (asymmetrical vocal fold vibrations with reduced amplitudes and mucosal wave reduction) demand particular attention because continuous VLS monitoring of these patients allows early diagnosis of vocal fold carcinoma [7, 11, 13, 15, 17].

The recognition of clinical manifestations and exposure to risk factors for the vocal fold carcinoma is important to primary care in order to promptly refer the patient to otorhinolaryngological examination and to establish a timely indication for microlaryngoscopy with biopsy [18–21]. VLS is a very important prompt indicator for microlaryngoscopy with biopsy as a gold standard procedure for the diagnosis of vocal fold carcinoma. During microlaryngoscopy, the patient must be under general anesthesia, which makes examination of the larynx mobility impossible [22, 23]. Also, vocal fold scaring after the biopsy is possible as the tissue does not have the ability to regenerate. According to the American Academy of Otorhinolaryngology and Head and Neck Surgery guidelines for dysphonia diagnostics, VLS enables good visualization of the larynx, which is important before performing microlaryngoscopy [24, 25, 26].

CONCLUSION

Results of this study implicate fundamental significance of VLS in the diagnosis of early glottic carcinoma as VLS precisely predicts early glottic carcinoma when an adynamic segment or the entire nonvibrating vocal fold is found.

Conflict of interest: None declared.

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Ендовидеостробоскопија у раној дијагностици карцинома гласница

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САЖЕТАК

Увод/Циљ Гласнице представљају најчешћу примарну локализацију ларингеалног карцинома. Напредак у дијагностици и терапији омогућио је бољу прогнозу за болеснике са раним карциномом глотиса.

Основни циљ нашег истраживања је да укажемо на значај ендовидеостробоскопије у раној дијагностици карцинома гласница.

Методе У проспективну контролисану студију укључено је 300 болесника са дисфонијом, хоспитализованих у терцијарној медицинској установи ради микроларингоскопије са биопсијом. Код свих болесника учињен је ендовидеостробоскопски преглед пре биопсије. Анализа ендовидеостробоскопског налаза је класификована према Хирану у четири стадијума, тако да адинамични сегмент и одсутне вибрације гласнице представљају стробоскопски налаз IV, суспектан за карцином гласнице. Према Љубљанској класификацији, хистопатолошки налаз подељен је на једноставну, абнормалну и атипичну хиперплазију и карцином *in situ*.

Резултати Анализа ендовидеостробоскопског налаза показала је да код 41,67% болесника (n = 125/300) постоје обострано присутне асиметричне и иррегуларне вибрације гласница, јако редукованог мукозног таласа (стробоскопски налаз III), док су код 17,33% (n = 52/300) болесника уочени адинамични сегмент и одсутне вибрације гласнице (стробоскопски налаз IV), суспектни на карцином гласнице. Хистопатолошком анализом, карцином гласница је верификован код 5,6% болесника са стробоскопским налазом III (n = 7/125) и код 26,92% болесника са стробоскопским налазом IV (n = 14/52). Налаз адинамичног сегмента и одсутних вибрација гласница има сензитивност 66,77% и специфичност 86,4% у детекцији карцинома гласница, са умереном дијагностичком прецизношћу (AUC = 0,844).

Закључак Ендовидеостробоскопија игра важну улогу у правовременој индикацији за микроларингостробоскопију са биопсијом у дијагностици карцинома гласница.

Кључне речи: ендовидеостробоскопија; карцином гласница; микроларингостробоскопија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

High-dose-rate endobronchial brachytherapy in the management of advanced lung cancer – comparison according to the presence of lung atelectasis

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SUMMARY

Introduction/Objective Locally advanced lung cancer is often accompanied by atelectasis of either a part or the entire lung. The aim of this study was to establish the benefits of brachytherapy on the patients' quality of life, the length of the progression-free survival (PFS), and the overall survival (OS) as related to the presence or absence of atelectasis after the applied treatment.

Methods The total of 100 patients with locally advanced lung cancer or endobronchial metastasis of other malignancy were treated with the high-dose-rate endobronchial brachytherapy (HDR-EBB) in 2017. For observing the patients' clinical characteristics, the PFS and OS, the patients were classified into four groups according to the presence of atelectasis before and after HDR-EBB.

Results After HDR-EBB alone or combined with other treatment modalities, a statistically significant symptom alleviation was registered for all the symptoms except cough ($p < 0.05$). The significantly highest PFS value was registered among the patients with atelectasis prior but not after HDR-EBB. The longest survival was registered in the patients who had atelectasis prior to, but not after HDR-EBB, as well as among the patients without atelectasis either before or after EBB.

Conclusion HDR-EBB is an efficient method that improved the quality of life of most patients. There were improved rates of re-aeration after HDR-EBB treatment alone and as a part of combined treatment. Re-aeration after EBB is a positive prognostic factor with respect to PFS and OS of these patients.

Keywords: atelectasis; brachytherapy; lung cancer; progression-free survival; survival

INTRODUCTION

Worldwide, lung cancer (LC) still has the highest incidence and mortality compared to other malignancies, with 2.1 million new LC cases and 1.8 million deaths predicted in 2018 [1]. Five-year survival rate is still low, it has been registered in only 19% of the cases, despite advances achieved in the fields of surgery, irradiation, and chemo treatment, as well as introduction of entirely new treatment modalities such as molecular and immunotherapy [2]. Among patients with diagnosed non-small cell lung cancer (NSCLC), 25–30% have either stage I or stage II of the disease according to the tumor, nodal, metastasis (TNM) classification, 30% have a locally advanced disease (TNM stage III), and the remaining 40–45% have distant metastases (stage IV). In LC after external beam radiotherapy (EBRT), local relapses of the disease are registered in almost 60–70% of the patients, and in 60% of the patients a fatal outcome occurred due to respiratory failure, obstructive pneumonia, and sepsis. Endobronchial brachytherapy (EBB) is an efficient method for palliative treatment in advance NSCLC resulting in improvement of the QoL in most patients [3, 4].

The total endoluminal obstruction induces atelectasis of the lung – segmental, lobar, or complete, and the resulting pneumonia with prominent symptoms such as dyspnea, elevated body temperature, hemoptysis, cough, suffocation. The bronchial obstruction type determines the optimal therapeutic regimen, and the endoluminal obstruction may be resolved by brachytherapy, laser therapy, photodynamic therapy or cryotherapy, while the extraluminal obstruction may be eliminated by external radiotherapy or stent placement [5]. Interventional bronchoscopy therapeutic procedures may result in a rapid alleviation of the symptoms and are often well tolerated, with minimal toxicity [4].

In locally advanced LC, brachytherapy is most frequently applied as palliative treatment procedure accompanied by other interventional bronchoscopy procedures. For high-dose-rate endobronchial brachytherapy (HDR-EBB), hospitalization is mostly unnecessary and the therapy is administered with a variation of fractionating modalities and dosage, depending on whether is intended for a curative or a palliative effect [4, 6]. EBB can be combined with other treatment modalities, including EBRT, chemotherapy, biological, or immune therapy [7, 8].

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In this study, we report our experience with HDR-EBB to assess its efficacy and tolerability in the treatment of patients with atelectasis caused by endobronchial metastatic tumors and LC.

METHODS

Material

Having obtained the approval for the research of the Board of Ethics of the Institute for Pulmonary Diseases of Vojvodina, a retrospective review of 100 patients (≥ 18 years old) with endobronchial (lung or metastatic) tumor was conducted. The patients had bronchoscopy-established advanced stage (IIIB and IV) LC, or a bronchial metastatic cancer from an extrapulmonary primary tumor. The patients with a "positive endobronchial status" (the tumor was seen in the trachea or the main bronchi) were diagnosed in the period from January 2017 to January 2018, giving a retrospective character to the study and enabling monitoring of the three-year survival.

Guided by bronchoscope, an endobronchial polytetrafluoroethylene catheter is induced into the tumor area. The position of the tumor regarding the catheter and the segment volume which is necessary to irradiate is measured by orthogonal X-ray imaging, based on which a radiation field is planned with calculation of dose distribution. Application dose is given in two fractions of 7 Gy, in weekly treatments. The dose is prescribed at 1 cm from the source axis. After connecting the catheter to the HDR brachytherapy machine, irradiation is conducted remotely after the loading technique, and a radioactive source (isotope Ir192) is placed in the vicinity of the tumor trough the catheter.

The OS assessment started from the moment of bronchial biopsy-established diagnosis, lasting to the end of the follow-up period or the date of death according to the Lung Cancer Registry of the Institute. The patients' identity was protected in strict accordance with the Declaration of Helsinki, seventh revision.

Statistical analysis

A total of 100 patients treated with brachytherapy were classified into two groups according to the presence or absence of lung atelectasis. The description within the groups was performed using absolute values and percentiles. The statistical analysis of the clinical features and treatment outcomes was performed by the Pearson χ^2 and the Fisher's exact test. The log-rank test was used to compare the overall survival (OS) and progression-free survival (PFS) outcomes between the groups. The OS and the PFS were compared using the median, with the monitoring period ≤ 36 months, and they were graphically presented using the Kaplan–Meier analysis and the MedCalc statistical software package (MedCalc Software Ltd, Ostend, Belgium), accepting $p < 0.05$ for the statistical significance level.

RESULTS

Patient and tumor characteristics

The total of 100 patients were treated with HDR-EBB at the Oncology Institute of Vojvodina, with a bronchologist of the Institute for Pulmonary Diseases of Vojvodina placing endobronchial catheters. Ninety-eight patients had a primary or recurrent disease of LC, and only two patients had endobronchial metastasis. The patients were classified into two groups: Group A – the patients who had atelectasis at the time of establishing the diagnosis, and Group B – the patients who did not have atelectasis at the time of establishing the diagnosis. The patient and tumor characteristics are summarized in Table 1. Group A included 47 patients, and Group B had 53 subjects. The mean age of the examined patients was 64 years. The youngest and the oldest patient were 44 and 84 years of age, respectively. There were 86 males and 14 females. In endoscopically visible bronchial cancer or bronchial infiltration, no statistically significant sex-related differences regarding the presence of atelectasis at the moment of diagnosing LC were registered. Neither were statistically significant European Cooperative Oncology Group performance status differences registered between the examined groups ($p = 0.196$). Regarding the histological tumor type, squamous lung cancer was most common, followed by adenocarcinoma, small cell and large cell lung cancer, while two patients had an endobronchial metastasis of the colon cancer. There existed a statistically

Table 1. Patient and tumor characteristics before high-dose-rate endobronchial brachytherapy related to the presence of atelectasis

Median age/ range (years)	64 (44–84)	with AT (A)	without AT (B)	p
Sex	Male	42 (89.36%)	44 (83.02%)	0.362[a]
	Female	5 (10.64%)	9 (16.98%)	
ECOG performance status	0 1 2 3	/ 34 11 1	/ 35 19 /	0.196[a]
Histology	Squamous ca.	33 (70.21%)	37 (69.81%)	0.205[a]
	Adenocarcinoma	7 (14.89%)	10 (18.87%)	
	Large cell ca.	1 (2.13%)	3 (5.66%)	
	SCLC	4 (8.51%)	1 (1.89%)	
	Metastatic	/	2 (3.77%)	
	Others	2 (4.26%)	/	
Site (endobronchial-positive finding)	Trachea	/	16 (30.19%)	0.001[a]
	Main br. R	15 (31.91%)	11 (20.75%)	
	Main br. L	22 (46.81%)	6 (11.32%)	
	Middle br.	2 (4.26%)	1 (1.89%)	
	Upper br. R	2 (4.26%)	3 (5.66%)	
	Upper br. L	2 (4.26%)	2 (3.77%)	
	Lower br. R	2 (4.26%)	2 (3.77%)	
	Lower br. L	/	4 (7.55%)	
	Br. intermedius	2 (4.26)	2 (3.77%)	
	Both sides	/	6 (11.32%)	
Total		47 (100%)	53 (100%)	

AT – atelectasis; R – right; L – left; ECOG – European Cooperative Oncology Group; SCLC – small cell lung cancer;
[a]Pearson's χ^2

significant difference (p = 0.001) regarding the localization of the bronchial tumor or bronchial involvement by the tumor: the positive endoscopy finding was most frequently obtained from the left main bronchus, then from the right main bronchus, and finally other endoscopy tumor localizations.

Treatment characteristics

HDR-EBB gave as a palliative and symptom-relieving method in all the patients. Of 100 examined subjects, 47 had atelectasis of a part or the entire lung at the moment of establishing the diagnosis, while 53 patients had no atelectasis. After the palliative EBB had been applied, either alone or in combination with other therapy modes, atelectasis was registered in 11 patients, while 89 patients were without atelectasis (Table 2). For this clinical treatment response, the difference was statistically significant (p = 0.022). EBB was administered alone in 26 patients, combined with EBRT in six patients, combined with chemotherapy in 23 patients, and combined EBRT with chemotherapy in 45 patients. There was no statistically significant difference between these therapeutic options in the "loss" of atelectasis following the treatment (p = 0.186). These results are reviewed in Table 3.

Palliation rate and clinical response

All the patients were evaluated for subjective symptom response summarized in Table 4. Analyzing the most common symptoms present at the moment of establishing the diagnosis on endoscopy and then after EBB alone or combined with other treatment modalities, a statistically significant symptom alleviation was registered for all the symptoms except cough.

Local control, time to progression, and overall survival

To assess the PFS and OS, all the patients were subdivided into four groups: Group I – patients with atelectasis prior to EBB, persisting after the treatment as well; Group II – patients with atelectasis prior to, but not after EBB; Group III – patients having no atelectasis prior to EBB, but developed it after the treatment, and Group IV – patients free of atelectasis before as well as after EBB. In all of the examined patients, the two-year PFS was 9%. One of the patients belonged to Group I [making 11.11% of the group (1/9)], four patients belonged to Group II [making 10.53% of the group (4/38)], none of the patients belonged to Group III, and four patients belonged to Group IV [making 7.84% of the group (4/51)]. The PFS median was 0, 10, 0, and 2 months in Groups I, II, III, and IV, respectively. The log-rank was 0.028, suggesting a different PFS in the examined groups; the significantly highest PFS value was registered among the patients with atelectasis prior but not after EBB (Group II), as well as in the patients free of atelectasis either before or after EBB – Group IV (Table 5, Figure 1). To evaluate the OS, the patients were classified into the same groups as for the PFS data. Of 100 patients, a 12-month OS was achieved by 44%, while a 24-month OS was achieved by 13% of the patients, one belonging to Group I, six to Group II, none to Group III, and six patients to Group IV. The total OS median was 10 months, the longest OS (12 months) was in Group II, and the shortest one (0) was in Group III. The log-rank was p = 0.002, suggesting there were statistically significant differences in the OS among the examined groups – the longest survival was registered

Table 2. Local control of the disease before and after high dose rate endobronchial brachytherapy related to the presence of atelectasis

		Atelectasis before TH		p
		Yes	No	
Atelectasis after TH	Yes	9 (19.15%)	2 (3.77%)	0.022[b]
	No	38 (80.85%)	51 (96.23%)	

TH – therapy;
[b]Fisher's exact test

Table 3. The presence/absence of atelectasis related to the treatment characteristics

Treatment	AT	Before EBB	After EBB	p
EBB alone	Yes	6	4	0.186[a]
	No	20	22	
EBB + EBRT	Yes	3	1	
	No	3	5	
EBB + CHT	Yes	12	1	
	No	11	22	
EBB + EBRT + CHT	Yes	26	5	
	No	19	40	

EBB – endobronchial brachytherapy; EBRT – external beam radiotherapy; CHT – chemotherapy
[a]Pearson's χ^2

Table 4. Symptom response

Symptom	Present	Before EBB	After EBB	p
Temperature* after TH	Yes	7	6	0.003[a]
	No	15	72	
Cough after TH	Yes	49	4	0.731[b]
	No	42	5	
Dyspnea after TH	Yes	49	1	0.001[b]
	No	37	13	
Hemoptysis after TH	Yes	5	6	0.025[a]
	No	15	74	

TH – therapy; EBB – endobronchial brachytherapy;
[a]Pearson's χ^2;
[b]Fisher's exact test;
*temperature > 38°C

Table 5. Progression free survival (PFS) and overall survival (OS) in lung cancer patients with and without atelectasis

Group	PFS (median) months	OS (median) months
Group I	0	4
Group II	10	12
Group III	0	0
Group IV	2	8
No accordance to AT	5	10
Log-rank	p = 0.0284	p = 0.0028

AT – atelectasis; Group I – patients with atelectasis prior to endobronchial brachytherapy; persisting after treatment as well; Group II – patients with atelectasis prior to but not after endobronchial brachytherapy; Group III – patients having no atelectasis prior to endobronchial brachytherapy but developed it after treatment; Group IV – patients free of atelectasis before as well as after endobronchial brachytherapy

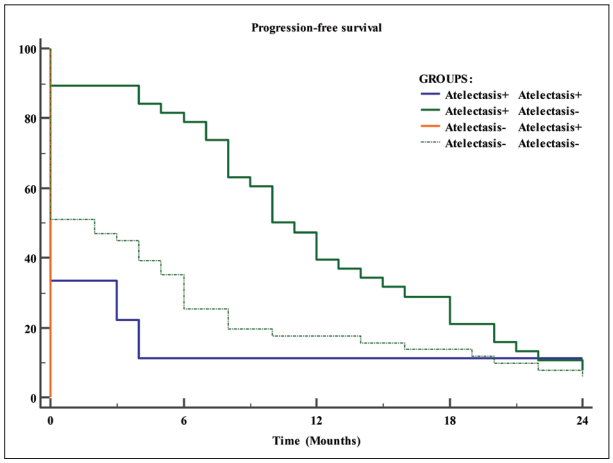

Figure 1. Progression-free survival in patients treated with high-dose-rate endobronchial brachytherapy according to the presence of lung atelectasis

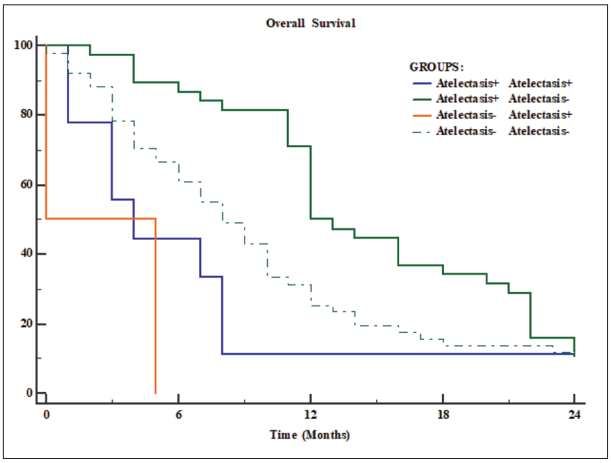

Figure 2. Overall survival in patients treated with high-dose-rate endobronchial brachytherapy according to the presence of lung atelectasis

in patients who had atelectasis prior to but not after EBB (Group I), as well as among patients free of atelectasis either before or after EBB (Group IV) (Table 5, Figure 2).

During the procedure, one patient had life-threatening hemoptysis but survived and was alive six months after therapy.

DISCUSSION

As 30% of LC patients have a locally advanced disease (stage III), and 40–45% have distant metastases (stage IV), palliative treatment procedures are probably a sole option for these patients. EBRT can also be a palliative treatment procedure affecting the tumor size, but its effect is rather slow and limited by the total radiation dose, and the maximal atelectasis regression achieved is 20% provided that other local interventional bronchoscopy procedures are not necessary [9]. Depending on the endobronchial tumor and the tumor compression type, brachytherapy may in some cases be the treatment of choice, either as a single therapy or combined with other interventional bronchoscopic procedures. Brachytherapy stops the obstruction process and removes atelectasis, improving patients' QoL [9]. Brachytherapy is not effective for acute and severe central airway obstruction (CAO) because it takes minimally three weeks for its effect [10]. Our study was aimed at establishing the presence of atelectasis of either the entire lung or its part, which was due to an intraluminal obstruction by the tumor or tumor-infiltrated bronchial mucosa, as well as the effects of EBB on the obstruction and atelectasis removal.

Several studies have reviewed different HDR-EBB regimens correlated to NSCLC stage, EBB fractionation modality mode, the number of installed catheters and delivered doses, as well as a clinical response. Bedwinek et al. reported on 60 patients who received HDR-EBB in three 6 Gy fractions (3 × 6 Gy), which resulted in a clinical improvement (76%), chest X-ray improvement (64%), bronchoscopy improvement (84%), with the median OS of 10 months [3]. Speiser and Spratling reported on 66 patients who received HDR-EBB in the dose of 3 × 10 Gy, registering a clinical and bronchoscopy improvement in 88% and 99%, respectively [4].

All the patients included in our study received HDR-EBB in two fractions of 7 Gy, the treatment was given weekly, in total of 14 Gy locally. In the patients who had the trachea bifurcation infiltrated, we installed two catheters bilaterally in both fractions.

Our examined sample of 100 patients included 86 males and 14 females at the mean age of 64 years. These clinical characteristics correlate to the reported studies of locally advanced NSCLC. Squamous lung cancer is the most common histological type of centrally located and endoscopy visible tumors. Proximal or CAO complicates 20–30% of LC cases and 40% of them originate from squamous NSCLC [11]. In our study, squamous lung carcinoma was diagnosed in 33 (70.21%) patients having atelectasis prior to treatment, and in 37 (69.81%) patients without atelectasis, exceeding the number reported by other authors, which is probably due to the high incidence of squamous LC in our region, as well as a high incidence of smokers among LC patients. After EBB had been applied as a palliative therapeutic procedure, either alone or combined with other treatment modalities, 11 patients had atelectasis and 89 did not. This clinical response was statistically significant ($p = 0.022$). Erickson et al. [12] reported a partial remission (atelectasis elimination) was achieved in 101 of their 188 examined patients, a minor response was registered in 25/188, no response in 29/188, while 33/188 patients developed progression in terms of atelectasis emerging in cases where it was formerly absent. Evaluating the applied treatment modalities, the best treatment response in terms of atelectasis elimination was achieved by EBB combined with a double-agent chemotherapy regimen and then by the EBB and EBRT combined. Mantz et al. [13] reported the best treatment response in terms of the local control of the endobronchial disease applying the treatment regimen with EBB followed by EBRT combined (EBB total dose 18 Gy in three fractions of 6 Gy in 4–7-day intervals

– the patients already treated with EBRT had a dose reduction to 50 Gy). However, the latest studies report that the published evidence did not provide conclusive evidence to recommend combined endobronchial and external-beam radiotherapy, EBB over external-beam, chemotherapy and neodymium-doped yttrium aluminum garnet laser treatment [6].

EBB later caused the effect on airway recanalization and also provides delayed spirometry improvement (forced expiratory volume in one second, forced vital capacity), pulmonary ventilation and perfusion and exercise tolerance of the five-minute walking distance [14]. A control of the symptoms, i.e., their elimination or improvement, is characteristic for all interventional airway recanalization treatment procedures, including EBB as well. In our study, analyzing the most common symptoms present at the moment of establishing the diagnosis and then after EBB alone or combined with other treatment modalities, a statistically significant symptom alleviation was registered for all the symptoms except cough. The presence of cough as the disease symptom may be explained by a definite damage of the tissue zones in the main airways, impossible to be entirely revitalized by palliative EBB. Several authors reported that a temporary dyspnea elimination may even result in a prolonged suffering from the patients' point of view [15]. It is therefore necessary to establish whether the patients' QoL will be clearly improved, as well as the survival benefit after interventional therapy in patients with inoperable malignant CAO. Most studies investigated dyspnea and performance status scores partially, but not the overall QoL [16, 17]. Neither did we investigate the QoL in our study, but we did investigate the performance status, obtaining a statistically significant improvement of our patients' performance status after the treatment.

Our study focused on the patients' survival related to the presence and "loss" of atelectasis after EBB. Statistically significant differences in the OS have been registered among the examined groups, that is – the longest survival was registered in the patients who had atelectasis prior to but not after EBB, as well as among the patients free of atelectasis either before or after EBB. In other studies which compared the OS according to the presence of atelectasis (with no interventional bronchoscopy procedures applied), the presence of atelectasis emerged as a positive prognostic factor, unlike the results of our study [18, 19].

Comparing our results to those obtained by other authors describing the application of palliative endoscopy procedures such as laser therapy, electrocautery, diathermy, electrocoagulation, phototherapy, cryotherapy, endobronchial stent insertion, and combinations of these techniques, similar results have been obtained regarding the OS of the patients with a CAO [20–23]. Future comparisons with stereotactic body radiotherapy and other ablative techniques are warranted to expand multi-disciplinary management options [24].

Although brachytherapy requires multidisciplinary coordination in a protected operating room or brachytherapy suite, patient sedation, bronchoscopy, and planning that increases the risk of exposure to patients and providers, radiotherapy remains one of the key treatment options for lung cancer in the era of the COVID-19 pandemic [23, 25].

CONCLUSION

Brachytherapy as a palliative interventional airway recanalization endoscopic treatment is a safe therapeutic tool that independently or in association with other therapeutic modalities leads to the improvement of patients' QoL suffering from locally advanced LC. Significant differences in the PFS and the OS have been registered among the examined groups, with the longest survival being registered in patients who had atelectasis prior to but not after EBB.

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Високодозна брахитерапија код узнапредовалог карцинома плућа – анализа присуства плућне ателектазе

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САЖЕТАК

Увод/Циљ Локално узнапредовали карцином плућа се често презентује ателектазом једног дела или целог плућног ткива.

Циљ ове студије је да се утврде утицај високодозне ендобронхијалне брахитерапије (*HDR-EBB*) на квалитет живота болесника, време до прогресије болести, као и укупно преживљавање у односу на присуство/одсуство ателектазе након терапијског третмана.

Методе Студија је обухватила 100 болесника са узнапредовалим карциномом плућа или метастазама лечених *HDR-EBB* током 2017. године. Како би се посматрале клиничке карактеристике, време до прогресије болести и укупно преживљавање, болесници су сврстани у четири групе у односу на присуство ателектазе пре и после *HDR-EBB*.

Резултати После самосталне *HDR-EBB* или у комбинацији са другим начинима лечења, утврђено је статистички значајано повлачење већине симптома, осим кашља ($p < 0,05$). Статистички значајно је продужено време до прогресије болести код болесника код којих је дошло до повлачења ателектазе након третмана ($p = 0,0284$). Најдуже укупно преживљавање је забележено код болесника код којих се после третмана повукла ателектаза ($p = 0,0028$), или који нису имали ателектазу ни пре ни после третмана.

Закључак *HDR-EBB* је ефикасан третман у побољшању квалитета живота болесника. Након самосталне брахитерапије или комбиноване са другим терапијским модалитетима уочено је значајно повећање степена аерације плућа. Повлачење ателектазе након брахитерапије је добар прогностички фактор, који доводи до продуженог времена до прогресије болести и укупног преживљавања.

Кључне речи: ателектаза; брахитерапија; карцином плућа; време без прогресије; преживљавање

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Cerebral venous sinus thrombosis associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection

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SUMMARY

Introduction/Objective Coagulopathy induced by severe acute respiratory syndrome coronavirus 2 infection (SARS-CoV-2) can be an underlying cause of cerebral venous sinus thrombosis (CVST), a less common type of stroke with a variable clinical presentation and high mortality rate. The objective of the paper is to present a series of CVST cases associated with SARS-CoV-2 infection.

Methods This retrospective study evaluated clinical, laboratory and radiological presentations, risk factors, barriers to diagnosis, treatment and outcome of patients with SARS-CoV-2 infection-induced CVST.

Results The study comprised six patients diagnosed with COVID-19-induced CVST during an 18-month period. The majority (66.7%) had no significant risk factors for developing CVST. The median time from the initial COVID-19 diagnosis to the onset of neurologic deficit was seven days (interquartile range 0.5–7 days). Clinical presentation comprised non specific neurological symptoms: headache (83.3%) and decreased consciousness (33.3%), together with elevated levels of D-dimer and inflammatory biomarkers. The transverse (n = 4, or 66.7%), superior sagittal sinuses (n = 3, or 50%) and sigmoid sinus (n = 2, or 33.3%) were most commonly affected. Five patients (83.3%) had minimal to no symptoms at discharge (mRS ≤ 2). In-hospital mortality in our current series was relatively high (16.7%).

Conclusion The high mortality rate of SARS-CoV-2-associated CVST urges clinicians to suspect CVST in patients with a history of COVID-19 infection presenting with non-specific neurological symptoms in order to provide proper treatment and prevent complications.

Keywords: COVID-19 coagulopathy; anticoagulation; stroke

INTRODUCTION

Cerebral venous sinus thrombosis (CVST) is a rare form of cerebrovascular incident (< 1%), induced by partial or complete occlusion of the dural venous sinuses and/or cerebral veins. Contrary to arterial stroke, it is more frequent in young adults, mostly affecting women [1]. Predisposing factors for CVST are numerous, but CVST associated with SARS-CoV-2 infection is a less known entity with no identifiable risk factors [2]. SARS-CoV-2 infection induces hypercoagulable state, comprising elevated D-dimer, fibrinogen level, fibrin/fibrinogen degradation product, antiphospholipid antibodies, and thrombocytopenia, which increases the risk of thrombus formation within the dural venous sinuses and/or cerebral veins [3, 4]. Clinical presentation varies, depending on the affected venous sinus and/or cerebral veins, presence of raised intracranial pressure (ICP) or extensive parenchymal damage. Non-specific clinical presentation of CVST urges clinicians to raise clinical suspicion and proceed with neuroradiological assessment. Management of CVST is based on early diagnosis with identification of thrombotic process, together with urgent conservative and endovascular treatment. Up to 80% of patients have a good outcome with a complete recovery. However, the outcome of a small proportion of patients (~13%) is poor (death or severe disability) [2].

The objectives of the present paper were to evaluate a case series of six patients regarding past medical conditions, risk factors, clinical and radiological presentation, barriers to diagnosis, treatment, and outcomes in COVID-19-induced CVST patients.

METHODS

This was a single-center retrospective study at an academic hospital: Emergency Center – Clinical Centre of Vojvodina, Novi Sad, Serbia. We extracted data on six CVST patients with COVID-19 from the hospital information system from March 6, 2020 to September 6, 2021. The individual case data comprised the following: patient demographics, comorbidities, risk

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factors, clinical presentations, National Institutes of Health Stroke Scale (NIHSS), Glasgow Coma Scale (GCS) score on admission and during the clinical course, location of thrombosis and brain lesions (radiological presentation), laboratory results (white cell count, absolute lymphocyte count, platelets, prothrombin time (PT), activated partial thromboplastin time (aPTT), C-reactive protein (CRP), D-dimer, fibrinogen, lactate dehydrogenase (LDH), and ferritin, treatment (anticoagulation, endovascular treatment, or neurosurgery) and inpatient mortality. SARS-CoV-2 infection was confirmed by the real-time reverse transcriptase polymerase chain reaction assay.

The radiologic diagnosis of acute CVST was confirmed by computerized tomography venogram (CTV) or magnetic resonance imaging (MRI) / magnetic resonance venogram (MRV) studies.

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

RESULTS

Patient demographics

Six patients with confirmed SARS-CoV-2 infection developed CVST. Most of the patients were female (n = 5, or 83.3%), with an average age of 44.3 ± 10.5 years old (Table 1).

Table 1. Patient demographics

Variable	Value
Age (years, mean ± SD)	44.3 ± 10.5
Sex – n (%)	
Male	1 (16.7%)
Female	5 (83.3%)

Table 2. Location of cerebral venous sinus thrombosis (computed tomography / magnetic resonance venogram)

Location	n (%)
Superior sagittal sinus	3 (50%)
Transverse sinus	4 (66.7%)
Sigmoid sinus	2 (33.3%)
Deep venous system	1 (16.7%)
Bilateral	2 (33.3%)
Multiple cerebral venous sinuses	3 (50%)

CVST risk factors

Two patients (33.3%) had common risk factors for CVST comprising an underlying pregnancy and malignancy. In most patients (66.7%), no known risk factors for CVST were identified.

Location of CVST

The most common localization of the thrombotic process was in the transverse (n = 4, or 66.7%) and the superior sagittal sinuses (n = 3, or 50%). CVST was also observed in the sigmoid sinuses (n = 2, or 33.3%) and in the deep venous structures (n = 1, or 16.7%). Half of our patients (n = 3, or 50%) developed central venous thrombosis involving multiple cerebral venous sinuses. Bilateral cerebral venous sinus involvement was present in two patients (33.3%), with the transverse sinuses being the most commonly involved (Table 2).

Intracranial hemorrhage was detected in one patient (16.7%) upon repeated CT brain imaging.

Presenting neurological symptoms

The median time to CVST clinical presentation from the initial COVID-19 diagnosis was seven days (interquartile range 0.5–7 days). Symptom occurrence was acute (< 48 hours) in 33.3% of the patients and subacute (> 48 hours to 30 days) in 66.7% of the patients.

Headache of various intensity, as the most common symptom of CVST, was identified in 83.3% of the patients. It was the only early neurologic manifestation in 33.3% of the patients. A CVST-associated headache was generally persistent and had positive correlation to disease severity. Headache occurred in all locations of cerebral venous occlusion, but was more pronounced in the sagittal sinus or straight sinus thrombosis. The headache of CVST was typically described as diffuse, progressive over time. The characteristics of headaches were diverse (Table 3), being unilateral or localized in 50% of the cases.

Focal neurological deficits occurred in three patients (50%). Unlike arterial ischemic stroke or intracranial hemorrhage, focal neurological signs did not occur so suddenly. The most common ones were presented as motor symptoms, followed by visual impairment and aphasia (Table 3).

Focal or generalized seizures occurred in two (33.3%) patients. Generalized seizures occurred in one (16.7%) patient, and focal seizures (simple partial seizures without generalization) in one (16.7%) patient (Table 3).

Altered consciousness, ranging from drowsiness to coma, was observed in two (33.3%) patients with CVST. Among patients with altered consciousness, one patient had moderate (GCS 8–10 points) and one patient had severely altered state of consciousness (GCS 3–7). Altered consciousness in combination with headache, focal neurological deficits and neuropsychiatric manifestations (confusion and amnesia) were most common in patients with deep venous system thrombosis (Table 3).

Neuro-ophthalmological symptoms were papilledema, loss of vision, and constriction of the visual field. Papilledema was identified in 66.7% of the patients with acute and subacute onset of the disease and was most often associated with headache.

Multiple cranial nerve involvement (cranial nerve palsy) occurred in one CVST patient (16.7%).

Psychosis in conjunction with focal neurological signs occurred in one patient (16.7%).

Table 3. Presenting neurological symptoms of cerebral venous sinus thrombosis and location of lesion

Presenting symptoms	Location of lesion	n (%)
Headache		5 (83.3%)
Migraine	Venous occlusion / focal lesion	
Raised ICP	Venous or sinus occlusion / large mass lesion	
Thunderclap	Venous occlusion / subarachnoid hemorrhage	
Focal neurologic deficit		3 (50%)
Hemiparesis	Infarction / hemorrhage / venous oedema	
Aphasia Sensory disturbance Inattention/neglect	Focal infarction / hemorrhage / superficial or deep venous system	
Cranial nerve palsy		
III, IV, V, VI VII, VIII IX, X, XI	Cavernous sinus Transverse/sigmoid sinus Posterior cavernous sinus / internal jugular vein / deep venous system	
Decreased consciousness		2 (33.3%)
Drowsiness, stupor, coma	Deep venous system / straight sinus	
Cognitive impairment		1 (16.7%)
Encephalopathy, disorientation, reduced concentration, amnesia	Deep venous system / temporal-parietal lesion (vein of Labbe) / seizures	
Seizures		2 (33.3%)
Focal	Focal infarction/hemorrhage	
Generalized	Focal infarction/hemorrhage / severely raised intracranial pressure	
Visual disturbance		4 (66.7%)
Reduced/altered visual field diplopia, papilledema	Raised intracranial pressure Posterior infarction/hemorrhage	

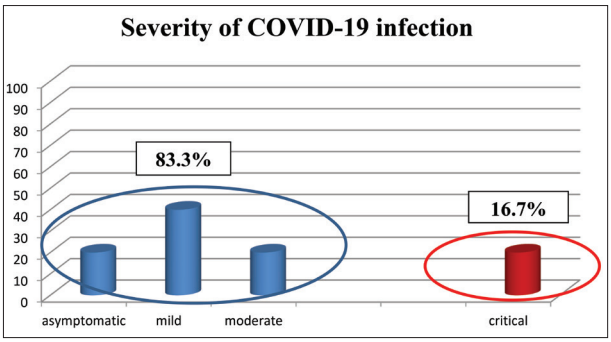

Figure 1. Severity of COVID-19 infection (patients with cerebral venous sinus thrombosis)

Severity of COVID-19 infection

Clinical presentations of the COVID-19 infection at the time of admission to the hospital was classified according to the World Health Organization guidelines into the following five categories: asymptomatic, mild, moderate, severe, and critical [5]. One patient (16.7%) presented with a critical form of the disease. The remaining four patients (83.3%) had asymptomatic to moderate disease (Figure 1).

Inflammatory biomarkers and coagulation studies

Inflammatory biomarkers such as CRP, ESR, ferritin, and LDH were elevated in most patients. Leukocyte count was not significantly elevated above the reference ranges. D-dimer values were significantly elevated. Values of the coagulation studies (aPTT, PT, INR and platelets) were within normal limits (Table 4).

Table 4. Inflammatory biomarkers and coagulation studies

Variable	Values
Inflammatory biomarkers	
CRP	48.2 ± 51.1 mg/L
ESR	51 ± 18.2 mm/hr
Ferritin	560.4 ± 191.3 ng/mL)
LDH	378.1 ± 166.8 units/L
WBC counts	8.8 ± 4.8 (thousand per uL)
Coagulation studies	
D-dimer	4612 ± 1121 ng/mL
aPTT	34.5 ± 14.1 s
PT	12.7 ± 1.1 s
INR	1.03 ± 0.1 s
Platelets	220.6 ± 112.9 × 10^9/L

CRP – C-reactive protein; PT – prothrombin time; aPTT – activated partial thromboplastin time; LDH – lactate dehydrogenase; ESR – erythrocyte sedimentation rate; INR – international normalized ratio

Table 5. Treatment modalities

Treatment modality	n (%)
Pharmacological treatment for COVID-19	6 (100%)
Therapeutic anticoagulation: LMWH and UFH	6 (100%)
Endovascular therapy (aspiration thrombectomy)	1 (16.7%)
Decompressive hemicraniectomy	1 (16.7%)
Anti-epileptic agents	2 (33.3%)
Therapy for elevated intracranial pressure: osmotic therapy, hyperventilation (pCO2 30–35 mmHg)	5 (83.3%)
Steroids (COVID-19 infection indications)	3 (50%)

UFH – unfractionated heparin; LMWH – low-molecular-weight heparin

Treatment modalities

Therapeutic modalities with anticoagulant therapy, treatment of Covid 19 infection, anticonvulsant medication and intracranial hypertension management are shown in Table 5. All patients received pharmacological treatment for SARS-CoV-2 infection recommended by the guidelines (World Health Organization: COVID-19 Clinical Management: Living guidance).

Outcomes

Objective quantification of the impairment caused by CVST (GCS and NIHSS) and mean length of hospital stay are shown in Table 6. Most patients (n = 5, or 83.3%) had good outcome with minimal or no symptoms (mRS ≤ 2) (Table 6). One patient with CVST and the risk factors of older age (> 50 years old), male sex, coma, mental status disorder, hemorrhage on admission CT scan, status epilepticus, deep CVST thrombosis, multiple involved venous sinuses, and severe to critical form of COVID-19 infection, did not respond to treatment and had a poor outcome.

Table 6. Patient outcomes

Outcomes	Value
Death, n (%)	1 (16.7%)
Discharged home (mRS)* ≤ 2, n (%)	4 (83.3%)
Mean length of hospital stay, days	17.2 ± 12.8
GCS (CVST diagnosis), median (IQR)	14 (12–15)
NIHSS (CVST diagnosis), median (IQR)	15 (7–15.5)
Days to clinical presentation of CVST, median (IQR)	1 (0–13)

GCS – Glasgow Coma Scale; CVST – cerebral venous sinus thrombosis; NIHSS – National Institutes of Health Stroke Scale; IQR – interquartile range

DISCUSSION

CVST associated with SARS-CoV-2 infection is a rare event, but it occurs 30–60 times more frequently compared to the non-COVID-19 population [6].

In our study, combination of CSVT and COVID-19 more frequently affected women (83.3%), alike the general population [7]. It occurs predominantly in young population, which was confirmed in our study, where the average age was 44.3 ± 10.5 years old.

Standard risk factors for CVST were not identified in most patients in our study (66.7%), which is in compliance with previous series (74%) [8].

Clinical presentation of CVST is not specific, based on the number and the localization of brain sinuses and veins, time of onset, adaptive mechanisms (collateral venous network), and the severity of brain parenchymal damage. Signs and symptoms comprise headaches (> 80%), seizures (~40%), hemiparesis (~40%), altered consciousness (15–20%), and papilledema (20–30%). The onset of symptoms was acute to subacute in about 80% of patients with CVST. As stated in the references, in our series, median time from clinical presentation to diagnosis of CVST was seven days [9]. Arterial stroke is not strongly associated with headache (25–30%), therefore severe headache in combination with stroke-like symptoms should draw attention to CVST. Seizures also occur more frequently in CVST than in the arterial stroke (40% *vs.* 6%). Focal neurological symptoms and signs are common. Rapid cognitive deterioration leading to drowsiness or coma is a typical clinical presentation of deep venous thrombosis associated with thalamic infarction [10].

Clinical presentation of SARS-CoV-2 infection was mild to moderate in most of our patients (83.3%). Therefore, the gravity of SARS-CoV-2 infection is not closely related to the gravity of CVST , which was also confirmed by other authors [11].

Radiological investigation is important for the diagnosis of CVST. Non-enhanced brain CT scan can confirm CVST in about 1/3 of the patients, based on hyperdensity within the venous sinus or the deep vein (dense triangle sign or the cord sign) [12]. CT can also detect ischemia, parenchymal or subarachnoid hemorrhage, or edema. CT venography has high sensitivity and specificity (95% and 91%). It identifies non-enhancement in thrombosed sinuses and veins and partial circumferential enhancement of thrombosed venous sinuses (empty delta sign) [13].

MRI and magnetic resonance venography (MRV) have higher diagnostic accuracy due to superior resolution and tissue characterization [14]. Additional value of MRV is the capability of confirming sinus thrombosis without administration of a contrast agent [15]. MRI is superior to other techniques in terms of parenchymal assessment involvement (ischemia, hemorrhage, oedema). Indications for digital subtraction angiography are inconclusive CTV or MRV findings, or suspected dural arteriovenous fistula [2].

The transverse sinus and the superior sagittal sinus are most commonly affected by CVST. Another typical finding is multiple vessels' thrombosis involvement, with a transverse sinus predominance [2]. "Deep" venous system affection leads to a poor overall prognosis and high mortality [10]. One of our patients (16.7%) had intracranial hemorrhage, identified by a follow-up CT brain scan. Findings of intracerebral hemorrhage in SARS-CoV-2 patients could indicate potential CVST.

SARS-CoV-2 is an underlying cause of a systemic inflammatory reaction, confirmed by our data on high levels of inflammatory biomarkers (ESR, CRP, ferritin, LDH) [16]. Extreme elevation of D-dimer confirms the assumption that COVID-19 infection may be related to systemic prothrombotic state [17]. In mild cases of CVST, D-dimer level can be normal, but it has a high negative predictive value for excluding CVST in patients with isolated headache. In general, no laboratory analysis can exclude CVST [18].

Treatment of CVST should be aimed at controlling the thrombotic process using anticoagulant therapy/endovascular procedures, treatment of the underlying cause (SARS-CoV-2 infection) and identified risk factors, seizure therapy, and treatment of intracranial hypertension.

Therapeutic doses of anticoagulant therapy should be administered as the basic treatment of CVST, regardless of the presence of intracranial hemorrhage [19]. In systemic VTE, studies suggest that low molecular-weight heparins

(LMWH) have shown better effectiveness compared to unfractionated heparins for the prevention of the thrombotic process progression with a lower risk of bleeding complications [20, 21]. After initial treatment with LMWH, long-term administration of vitamin K antagonists (warfarin) should be used to continue anticoagulant CVST therapy. Recommended durations of the chronic oral anticoagulant treatment are 3–6 months in provoked CVST, 6–12 months in unprovoked CVST, and potentially lifelong in recurrent CVST, CVST associated with venous thromboembolism, or CVST associated with thrombophilia [2].

The most common cause of death in patients with CVST is transtentorial herniation due to raised ICP [9]. Management of elevated ICP comprises osmotic therapy (mannitol), hyperventilation (pCO_2 30–35 mmHg) and head elevation. Carbonic anhydrase inhibitors (acetazolamide) could be useful in patients with severe headaches or visual impairment [2]. Decompressive craniectomy allows the swollen brain to expand and could favor collateral vein drainage in CVST by reducing ICP [22]. Steroid use is not recommended as it is associated with a poorer prognosis in CVST, even if a parenchymal brain lesion is present, unless the presence of the underlying condition, such as COVID-19 infection, require its administration [23].

Indications for endovascular treatment (endovascular thrombolysis or mechanical thrombectomy) are progression of the thrombotic process despite the use of anticoagulant therapy, clinical progression of the disease despite the use of anticoagulant therapy, and in patients with contraindications for the anticoagulant therapy [24].

Antiepileptics are prescribed to control seizures and should not be used routinely for prophylactic purposes [2]. There is no evidence on the optimal duration of the treatment. For seizures associated with oedema, infarction, or hemorrhage, the treatment should be continued for at least one year [25].

In-hospital mortality in our current series was relatively high (16.7%), similar to data from previous studies [26, 27]. Mortality rate is significantly higher compared to non-COVID-19 CVST populations (2–8%), suggesting that COVID-19 CVST patients have a poorer prognosis [28]. Factors predictive of poor prognosis include the following: older age (> 40 years old), male sex, coma, mental status disorder, hemorrhage on admission CT scan, status epilepticus, deep CVST thrombosis, multiple involved venous sinuses, and severe to critical form of the COVID-19 infection. Approximately 80% of the patients have good prognosis (mRS of 0–1), but they usually have consequences in the form of symptoms of depression or anxiety that affect their work ability [11].

Limitations of this study are related to the small statistical sample, which is a reflection of the small number of patients with COVID-19 infection-associated CVST. Given the rapidly growing number of patients with COVID-19 infection worldwide and the severity of CVST as a complication, it is crucial to define their correlation, clinical manifestations, diagnostic-therapeutic protocols, and treatment outcome. Available studies show great diversity in study design, imaging methods, reference standard, patient selection, and sample size, thus they are not feasibly comparable. Larger studies are needed to define more reliable conclusion about this significant health problem.

CONCLUSION

The high mortality rate and significant disability of the most productive population with COVID-19 infection-associated CVST obliges a high index of CVST suspicion in patients with even mild COVID-19 infection with non-specific neurological symptoms, to ensure early diagnosis, application of the most effective individually tailored treatment, and to prevent complications.

Conflicts of interest: None declared.

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Тромбоза церебралних венских синуса повезана са инфекцијом *SARS-CoV-2*

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САЖЕТАК

Увод/Циљ Вирусном инфекцијом САРС корона 2 (*SARS-CoV*-2) индукована коагулопатија може довести до тромботских компликација као што је тромбоза можданих венских синуса (ТМВС), ређи тип можданог удара са различитом клиничком презентацијом и високом стопом смртности. Циљ рада је презентација серије случајева ТМВС повезаних са инфекцијом *SARS-CoV*-2.

Методе Овом ретроспективном студијом евалуирани су клиничка, лабораторијска и радиолошка презентација, фактори ризика, проблеми у дијагностици, третману и исходу оболелих од ТМВС удружених са инфекцијом *SARS-CoV*-2.

Резултати Студија је обухватила шест болесника код којих је током периода од 18 месеци дијагностикована ТМВС удружена са инфекцијом *SARS-CoV*-2. Већина испитаника (66,7%) није имала значајне факторе ризика за развој ТМВС. Просечно време јављања неуролошког дефицита од иницијалне дијагнозе инфекције ковидом 19 било је седам дана (интерквартилни опсег 0,5–7 дана). Болесници су имали неспецифичне неуролошке симптоме као што су главобоља (83,3%) и поремећај свести (33,3%), уз повишен ниво Д-димера и инфламаторних биомаркера. Најчешћа локализација тромбозе била је трансверзални (*n* = 4 или 66,7%), горњи сагитални синус (*n* = 3 или 50%) и сигмоидни синус (*n* = 2 или 33,3%). Пет болесника (83,3%) отпуштено је кући са минималним симптомима или без њих. Морталитет у нашој серији је био релативно висок (16,7%).

Закључак Висок морталитет болесника са ТМВС код инфекције вирусом ковид 19 захтева велики опрез на присуство ТМВС код оболелих са неспецифичним неуролошким симптомима, у циљу адекватног терапијског третмана и превенције компликација.

Кључне речи: ковид 19 коагулопатија; антикоагулантна терапија; мождани удар

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Oral manifestations and rehabilitation of a patient with *osteogenesis imperfecta*

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SUMMARY

Introduction *Osteogenesis imperfecta* is a rare heritable connective tissue disorder characterized by increased fragility of the bony tissue. The incidence of orofacial alterations associated with *osteogenesis imperfecta* is variable and includes *dentinogenesis imperfecta*, malocclusions, hypoplasia of the jaws, delayed dental development and structural abnormalities of the teeth.

Case outline A 22-year-old female was referred to the Clinic for Pediatric and Preventive Dentistry for dental treatment. Enlarged head, triangular-shaped face, mandibular prognathism with excessive maxillary hypoplasia, lowered vertical occlusal dimension were present features. The intraoral findings included dentinogenesis imperfecta with Kennedy's class IV in the upper jaw and class II in the lower jaw. Panoramic radiograph revealed abnormalities in the crown and root shape, obliteration of the pulp chamber and severe deficiency of alveolar bone mass. Overall treatment involved five phases: I – preventive and prophylactic treatment, II – direct restauration of five teeth with glass ionomer cement, III – extraction of severely damaged teeth, IV – prosthodontic rehabilitation with removable partial dentures, V – maintenance and follow-up phase.

Conclusion Low prevalence and wide variety of signs and symptoms make dental treatment of *osteogenesis imperfecta* overly complex and challenging. Nevertheless, it is essential to improve craniofacial and dental function along with facial aesthetic.

Keywords: *osteogenesis imperfecta*; rare diseases; *dentinogenesis imperfecta*; partial dentures

INTRODUCTION

Osteogenesis imperfecta (OI) is a rare heterogeneous group of connective tissue disorders characterized by increased fragility of the bony tissue.[1] Its estimated frequency in the general population is about 1 in 15,000 to 20,000 newborns [2, 3]. Most patients have dominant mutations in one of two genes, *COL1A1* and *COL1A2*, which code the collagen type I synthesis [4, 5]. The genetic database has been updated with 18 gene mutations reported in a recent review [6]. These mutations lead to quantitative or qualitative changes in type I collagen, the substantial structural protein of bone and dentin. Consequently, the quality of the osseus tissue is reduced and bones are more fragile and prone to fractures. These multiple fractures could follow minor trauma or sometimes occur spontaneously [7]. In addition to numerous fractures, patients might display short stature, hearing impairment, blue sclera, skeletal deformities that affect craniofacial structures such as triangular facial form, large head size and soft calvaria [8]. The incidence of orofacial alterations associated with OI is variable and includes *dentinogenesis imperfecta* (DI), maxillary hypoplasia, skeletal class III deformity, crossbite, open bite, hypodontia/oligodontia and delayed dental development [9, 10, 11]. Malocclusions can impair daily activities, such as chewing and speaking, which has negative impact on the quality of life and serious psychological and social implications [12]. Also, affected teeth might have crowns with a bulbous structure, constriction in the cementoenamel junction, irregular root morphology, enlarged pulp chamber as well as pulp stones and obliterations [13, 14].

OI has diverse clinical expression, varying from very mild to severe with perinatal lethality [15]. Based on clinical findings, Forlino and Marini [16] and Basel and Steiner [17] described four types of OI and since than its classification has been continuously updated (Table 1).

To our knowledge, there are not many cases of prosthodontic rehabilitation of younger patients with OI reported in the literature. This rare disease is complex and requires multidisciplinary approach and medical expertise. The aim of this study was to present rare case of a patient with severe deforming type of OI and its dental treatment.

CASE REPORT

A 22-year-old female was referred to the Clinic for Pediatric and Preventive Dentistry, School of Dental Medicine, University of Belgrade for dental treatment. She had been diagnosed

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Table 1. Classification of *osteogenesis imperfecta*

Type	Inheritance	Gene	Clinical feature
I	AD	*COL1A1*, *COL1A2*	Blues sclera, normal stature, fractures, hearing loss, presence of DI rare
II	AD	*COL1A1*, *COL1A2*	Perinatal lethal, blue-grey sclera, small for gestational age, respiratory distress, limb deformities, "frog leg" positioning, soft calvarium
III	AD	*COL1A1*, *COL1A2*	Severe phenotype, short stature, multiple fractures, progressive deformities, may have DI, adolescent onset hearing loss
IV	AD	*COL1A1*, *COL1A2*	Milder than OI III, typically ambulatory, DI is common, adult-onset hearing loss, normal-grey sclera
V	AD		Mild to moderate, calcification of the interosseus membrane, radial head dislocation, hyperplastic callous formation
VI	AR	*SEFPINF1*	DI absent, like type III
VII	AR	*CRTAP*	Overlap with types II and III, milder forms also documented
VIII	AR	*LEPRE1*	Overlap with types II and III, milder forms also documented

AD – autosomal dominant; DI – *dentinogenesis imperfecta*; AR – autosomal recessive; CRTAP – cartilage-associated protein

with OI two days after birth. Postnatal skull radiography revealed constitutional bone fragility and decreased mineralization and radiograph of the upper limbs showed left ulnar fracture with present mild angulation. Further examinations indicated that it was OI type III.

Her medical history revealed that she had multiple fractures of the upper and lower extremities during childhood, as well as skeletal deformities and bisphosphonate treatment. She had common craniofacial features including bluish sclera, disproportionally enlarged head compared to the body, triangular-shaped face, spine deformity.

Clinical and radiographic examinations were performed to obtain a comprehensive evaluation of the maxillomandibular complex. Mandibular prognathism with excessive maxillary hypoplasia was noted. The facial appearance was irregular with disproportionate inferior third, and compression of the middle third. Consequently, vertical occlusal dimension was lowered.

The intraoral examination revealed DI. In the upper jaw, the frontal segment was edentulous and in posterior segments the existing teeth had caries lesions and direct restorations. In the lower jaw, lateral incisors, canines, left first premolar and right second molar were present and had brown opalescent hue. Furthermore, gingival recession was found in the buccal region of both lower lateral incisors, and the rest of her teeth showed excessive inclination of the crown and rotation. Posterior segments of alveolar mandibular ridge were underdeveloped and thin (Figure 1).

Panoramic radiograph was evaluated regarding abnormalities in crown and root shape, anomalies od pulp chamber and structure of the jaws. In both maxilla and mandible, severe deficiency of alveolar bone mass was present. Affected teeth had bulbous shaped crowns with constricted cementoenamel junction, short roots, and complete obliteration of the pulp chamber (Figure 2).

Considering the patient's medical history and complexity of the condition, a therapy was based on minimally invasive dental procedures with minor trauma. The patient's expectations were analyzed, and various treatment options were discussed. Overall treatment involved five phases: I – preventive and prophylactic treatment, II – direct restorations of teeth 14, 15, 17, 24, 25, 26 with glass ionomer cement, III – extraction of teeth 23, 27, 47 under

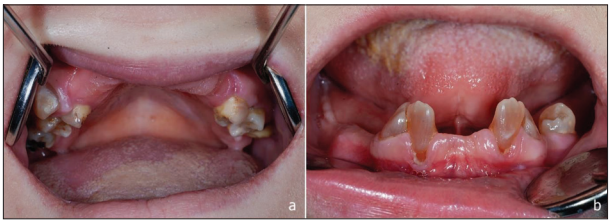

Figure 1. Clinical examination; intraoral photographs of the: a – maxillary, b – mandibular arch

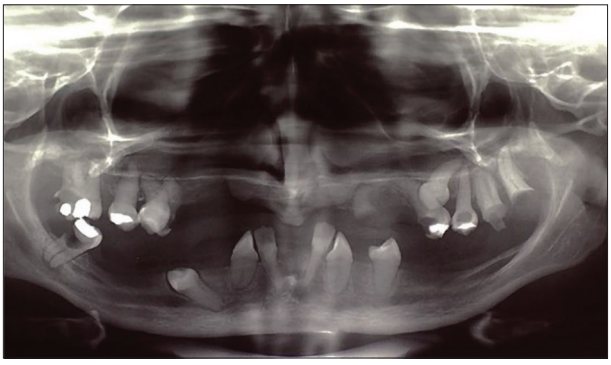

Figure 2. Radiographic evaluation: panoramic view showing irregular crown and root morphology of the present teeth; teeth with caries lesions and lower right second molar with periradicular radiolucency and a large loss of the crown; a residual root in the region of the upper left lateral incisor; deficiency of alveolar bone mass in both jaws

local anesthesia, IV – prosthodontic rehabilitation with removable partial dentures, V – maintenance and follow-up phase.

Prosthodontic rehabilitation of the patient started after extraction wounds had healed. Preliminary impressions were made for both arches using irreversible hydrocolloid impression material and study casts were obtained. Custom trays were made and used for definitive impressions. Occlusal rims were fabricated on the final casts and used to record maxillo-mandibular relationships. Adequate function, patient's facial characteristics and aesthetic and muscular tolerance were evaluated to determine the optimal vertical occlusal dimension. The waxed-up dentures were placed and evaluated in the mouth of the patient. After this phase, the dentures were finished, polished and after occlusal adjustment they were delivered to the patient (Figures 3 and 4). Also, she was trained how to maintain

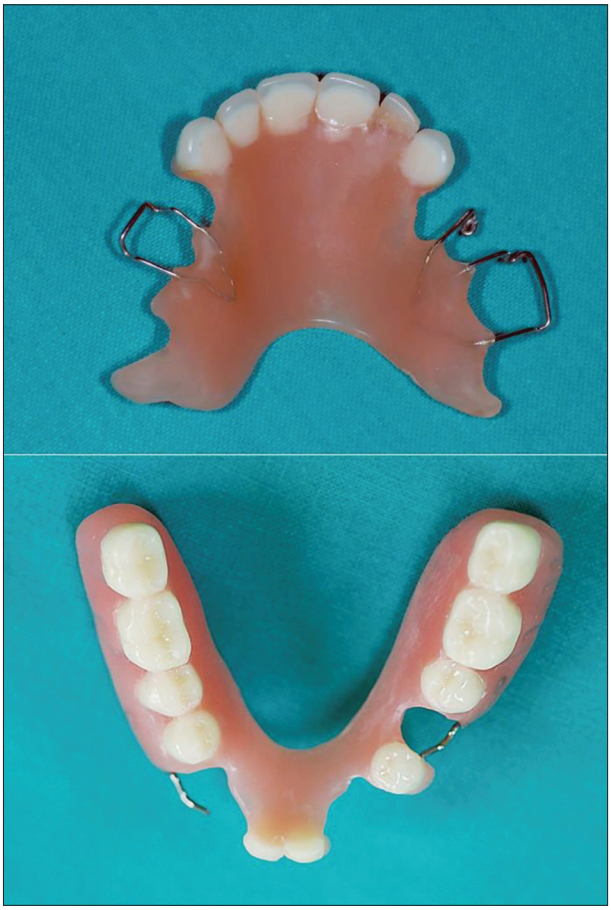

Figure 3. Maxillary and mandibular partial removable dentures

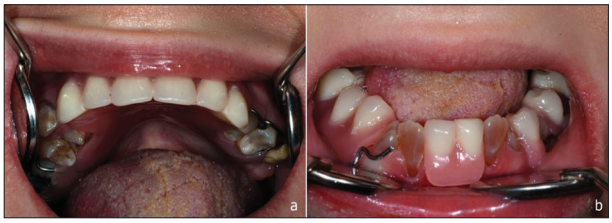

Figure 4. Intraoral photographs with adjusted partial dentures of the a – maxillary, b – mandibular arch

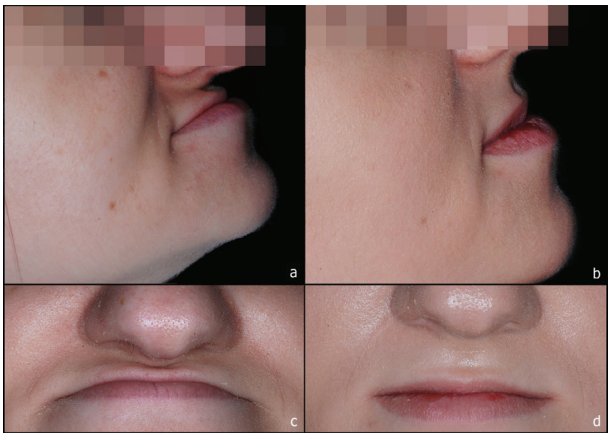

Figure 5. a – initial extraoral profile view, mandibular prognathism with maxillary hypoplasia; b – facial appearance after prosthodontic rehabilitation and restored vertical occlusal dimension; c – labial philtrum and upper lip before the prosthodontic treatment; d –after the treatment

proper oral and denture hygiene. An appointment was scheduled after a week for final adjustments and, after that, she was examined after three, six, and 12 months. She was satisfied with the functional improvement and with the aesthetic outcome of the treatment (Figure 5). The removable partial dentures did not need realignment after a year.

The present work was approved by competent ethics committee and conforms to the legal standards. Written informed consent for participation and publication, including clinical details and accompanying images, was obtained from the patient.

DISCUSSION

OI is a rare disease in which all parts of the body containing collagen type I can be affected, including skeletal system, dentin, dermis, tendons, organ capsules, fascia, meninges, cornea and sclera [18]. Literature data suggests that craniofacial and dental abnormalities are common findings [14, 19, 20]. The diagnostic procedure involves analysis of complete medical and family history, clinical examination and dental radiography. Previous studies suggested that multidisciplinary approach was needed to ensure accurate diagnosis and adequate treatment procedures [21]. Furthermore, dental team should include a pediatric dentist, a prosthodontist, a periodontist, an oral surgeon and an orthodontist.

Authors stated that OI type III was the most severe form in children which survive the neonatal period [16, 22]. These patients, along with OI type IV, require special dental care from the primary dentition [21]. Malocclusions are frequent finding in OI patients, especially class III [8, 23]. The malocclusions are caused by maxillary hypoplasia, mandibular prognathism or a combination of both factors. In addition, abnormal bone growth, posture, head size might be contributing factors to the development of malocclusions, which may become more serious with time [20]. Malocclusions can impair everyday activities such as chewing and speaking and consequently have negative impact on the quality of life, which was one of the main concerns of our patient [12, 24].

Another manifestation is DI which prevalence varies by OI type, from 21% to 73%, as reported in literature [13]. Bulbous crowns, short roots, obliteration of the pulp chamber, as seen in our patient, can compromise some dental procedures [25].

Intravenous bisphosphonates (BPs) are the primary treatment of children with moderate to severe OI. The main mechanism of their action is inhibition of osteoclast function and bone resorption. The effect of BPs therapy on the dental tissues is still unclear [26]. One of the concerns is development of bisphosphonate related osteonecrosis of the jaws following simple teeth extractions [25]. Studies reported that no complications had been observed after extractions of the primary teeth in children with BPs treatment [21, 27]. In the present case report, the patient did not have any complications in the healing process after extraction of the permanent teeth.

Individuals with OI have disturbances in organic and mineral bone components and altered biomechanical characteristics resulting in brittleness of bones. Moreover, it is followed by insufficient amount of bone, the cortical thickness and decreased amount of trabecular bone [28]. Malmgren et al. [14] reported that individuals with OI had a high prevalence of missing teeth, with a predilection for the posterior regions of the jaws. Panoramic radiograph of our patient revealed underdeveloped upper and lower jaw. Additionally, she has multiple teeth missing and consequently unpreserved vertical occlusal dimension which made normal functioning, especially eating, exceedingly difficult. To establish physiological function, preserve alveolar bone and achieve acceptable facial aesthetic, we carefully planned rehabilitation of the orofacial system [1]. Severe type of OI, combined with potential complications and patients' rejection of orthognathic surgical procedure, based the therapeutic strategy on minimally invasive treatment. Due to our patients' lower financial status, it was determined that the therapy included partial dentures was the best option.

The dental management of OI patient primarily depends on medical history, patient's age and needs, social and economic circumstances. The low prevalence and wide variety of signs and symptoms of OI, make the dental procedure complex and challenging. All present limitations and possible complications must be taken into consideration. However, the main goal is to improve craniofacial and dental function along with facial aesthetic. In this case report, functional and aesthetic rehabilitation was achieved, and the patient was successfully adapted to partial dentures.

Conflict of interest: None declared.

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Оралне манифестације и рехабилитација пацијента са болешћу *osteogenesis imperfecta*

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САЖЕТАК

Увод *Osteogenesis imperfecta* представља ретко, наследно обољење везивног ткива које карактеришу крхке кости склоне фрактурама и прогресивни коштани деформитети. Неке од орофацијалних манифестација су *dentinogenesis imperfecta*, малоклузије, хипоплазија вилица, закаснели развој зуба и структурне аномалије зуба.

Приказ болесника Двадесетдвогодишња болесница је имала карактеристичне промене: увећану главу, троугласти облик лица, мандибуларни прогнатизам са израженом хипоплазијом горње вилице и сниженом вертикалном димензијом оклузије. Клиничким прегледом утврђена је *dentinogenesis imperfecta*, а на ортопантомограму уочене су аномалије облика крунице и корена зуба, облитерација пулпне коморе и недостатак алвеоларне коштане масе. Рехабилитација је обухватила неколико фаза: 1 – превентивне и профилактичке мере; 2 – рестаурација каријесних лезија; 3 – екстракција зуба; 4 – протетска рехабилитација; 5 – контролни прегледи.

Закључак Стоматолошки третман овог комплексног ретког обољења захтева детаљну анализу и мултидисциплинарни приступ како би се постигла рехабилитација оралних функција, побољшала естетика лица, а тиме и квалитет живота пацијената.

Кључне речи: *osteogenesis imperfecta*; ретке болести; *dentinogenesis imperfecta*; парцијалне протезе

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Forgotten cause of severe hyponatremia

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SUMMARY

Introduction Sheehan syndrome is (pan)hypopituitarism because of postpartum pituitary infarction due to massive obstetrical hemorrhage. Enlargement of the pituitary gland, smaller sellar region, disseminated intravascular coagulation or autoimmunity are predisposing factors. The absence of lactation after labor and the inability to resume the menstrual cycle later are presenting symptoms. Some patients with Sheehan's syndrome have a sudden onset of severe hypopituitarism immediately after labor, most often in the form of severe hyponatremia. Central adrenal insufficiency is the most usual cause of hyponatremia, although in some cases the syndrome of inappropriate antidiuretic hormone secretion has been also described.

Case report A 39-year-old female patient was admitted to the Intensive Care Unit due to severe hyponatremia with neurological symptoms (Na 103 mmol/L, Cl 72 mmol/L, K 3.7 mmol/L), and absence of lactation. Previously, on the sixth postpartum day, she was admitted to the Obstetrics and Gynecology Clinic due to severe headache, nausea, vomiting, and blurred vision. The symptoms persisted since labor, which was complicated with severe hemorrhage (1000 ml) due to obstetric complications. Treatment began with the 3% hypertonic saline solution with restriction of fluid intake. In regard to panhypopituitarism, replacement therapy with hydrocortisone and levothyroxine was initiated. Diabetes insipidus was excluded. Growth hormone replacement therapy and combination of progesterone and progestogens was started during follow-up.

Conclusion Early diagnosis of Sheehan's syndrome is essential. Pituitary insufficiency in these patients has a great diversity in presentation, that can sometimes result in coma and death.

Keywords: postpartum hemorrhage; Sheehan syndrome; panhypopituitarism

INTRODUCTION

Hyponatremia defined as sodium level < 135 mmol/l is a frequent electrolyte disorder, and it can be found in patients with endocrinopathies [1]. In hospital setting, the incidence of hyponatremia is as high as 30% [2, 3]. Most commonly seen endocrinopathies together with euvolemic hyponatremia are the syndrome of inappropriate antidiuretic hormone secretion (SIADH), adrenal insufficiency and hypothyroidism [3]. Sheehan syndrome (ShS) is (pan) hypopituitarism on the grounds of postpartum pituitary infarction due to massive obstetrical hemorrhage. Signs and symptoms of ShS are mild and nonspecific, so the diagnosis can be easily missed or delayed [4]. Nationwide retrospective study in Iceland found the incidence of ShS of 5.1 per 100,000 females, while according to the KIMS database (Pfizer International Metabolic Database) the incidence of ShS was found to be 3.1 in 1034 patients with growth hormone deficiency. Occurrence of ShS in developed countries is estimated to be exceedingly rare due to highly developed obstetrical care [5]. Here we present a case of acute onset of hyponatremia in a patient who experienced hemorrhage during labor.

CASE REPORT

A 39-year-old female patient was admitted to hospital five days after parturition because of altered sensorium. Five days earlier she had given birth to a healthy newborn, but the labor had been complicated with extensive obstetrical hemorrhage. On admission, the patient was adynamic and confused, with signs of anemia but without signs of obstetrical bleeding. She reported that lactation was not established. She did not have polyuria or polydipsia. Blood was drawn for initial laboratory tests; the results are shown in Table 1. Due to severe overt hyponatremia, she was treated with 3% saline solution and further investigation was indicated. The results of endocrinological tests are shown in

Table 1. Initial blood results

Parameter	Value
Sodium (mmol/l)	103
Potassium (mmol/)	3.7
Chlorine (mmol/l)	72
Hemoglobin (g/l)	105
RBC ($\times 10^{12}$)	3.7
WBC ($\times 10^{9}$)	11.06
Hematocrit (%)	27.9
CRP (mg/L)	29.4

RBC – red blood cells; WBC – white blood cells; CRP – C-reactive protein

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Table 2. Endocrinological test results

Endocrinological test	Value
FSH (IU/L)	< 0.1
LH (IU/L)	< 0.5
PRL (ng/mL)	12.2
ACTH (pg/mL)	3
hGH (ng/mL)	0.12
IGF-1 (ng/mL)	134.8
Cortisol 8 a.m. (nmol/L)	14.5
24-hour urine sodium excretion (mmol/day)	83
Anti-TPO Ab (IU/ml)	1.4
Anti-Tg Ab (ng/ml)	18.6

FSH – follicle-stimulation hormone; LH – luteinizing hormone; PRL – prolactin; ACTH – adrenocorticotropic hormone; hGH – human growth hormone; IGF-1 – insulin-like growth factor 1; Anti – TPO Ab – thyroid peroxidase antibody; Anti – Tg Ab – antithyroglobulin antibodies

Table 2. Endocrinological testing showed panhypopituitarism. Parenteral hydrocortisone was introduced, and after a few days, levothyroxine was added. All the signs and symptoms of severe hyponatremia resided after the therapy and did not appear again after the hydrocortisone initiation. Magnetic resonance imaging of the pituitary gland was done, and it revealed enlarged pituitary with apoplexy without bleeding (Figure 1). On subsequent ambulatory check-ups, panhypopituitarism persisted, and a replacement dose of estrogen and progesterone was given, along with growth hormone therapy initiation. So far, we have been following this patient for three years and panhypopituitarism seems to be definite. She has been feeling well and has been asymptomatic.

Written consent for the publication of this article was obtained from the patient.

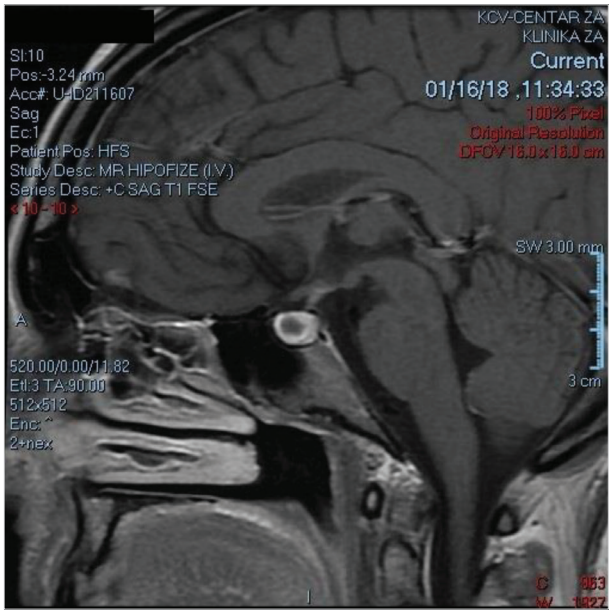

Figure 1. Enlarged pituitary with apoplexy without bleeding

DISCUSSION

ShS can be a life-threatening condition thus making a delay in diagnosis detrimental. It emerges because of extensive postpartum hemorrhage which leads to pituitary hypoperfusion and necrosis [6, 7]. Nowadays, ShS diagnosis is rare in developed countries, but in under-developed and developing countries it can be seen more often. With improving obstetrical care, the incidence of ShS is less frequent, but more than 40 years ago, the prevalence of this condition was around 100–200 per 100,000 females [8]. Miljić et al. [9] presented a retrospective study on 260 patients with hypopituitarism treated at their specialized endocrinological unit during a decade, and among those patients, two had ShS. Due to the rarity of this condition, nowadays, in countries with well-developed health care the data regarding the ShS incidence is not fully established [4, 8, 10]. Signs and symptoms of ShS are nonspecific and gradual in onset. The time from onset to the diagnosis is one month to 27 years [11, 12, 13]. Our patient had the abrupt onset of signs and symptoms of severe overt hyponatremia due to central adrenal insufficiency. Earlier reports of deaths due to diminished cortisol level in unrecognized partial or complete hypopituitarism after labor emphasize the need to reintroduce ShS as possible cause of rapid onset of worsening state in females after delivery [14, 15, 16]. Hyponatremia in ShS is mainly the consequence of central adrenal insufficiency i.e., the lack of cortisol. It is known that SIADH is the most common reason for euvolemic hyponatremia. There are clear diagnostic criteria for SIADH, nevertheless it is not always the case that the central adrenal insufficiency is priorly excluded [17, 18, 19]. Further contribution to the severity of hyponatremia comes from hypothyroidism as a part of hypopituitarism in the presented patient. Hyponatremia in isolated hypothyroidism is rare, and mostly the consequence of primary hypothyroidism. Proposed mechanism for the hyponatremia in hypothyroid patients is the elevation of antidiuretic hormone together with mild renal impairment [20–23]. Symptomatic hyponatremia should be urgently solved with intravenous 3% saline solution according to current guidelines, having in mind that too fast elevation of sodium can result in pontine myelinolysis [1, 24, 25, 26]. Timely diagnosis of ShS is needed in order to initiate adequate hormonal supplementation, and the hydrocortisone and levothyroxine are of vital significance.

ShS is a rare condition in developed countries and the significance of the risk it carries might be neglected. Central adrenal insufficiency can be component of ShS, thus contributing to the severity of syndrome. With timely diagnosis and adequate therapy, significant morbidity and, in some cases, mortality might be avoided.

Conflict of interest: None declared.

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Занемарени узрок хипонатремије тешког степена

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САЖЕТАК

Увод Шиханов синдром настаје услед исхемијске некрозе хипофизе због постпарталног крварења, а карактерише га различит степен хипопитуитаризма. Повећан волумен хипофизе, мања селарна регија, дисеминована интраваскуларна коагулација или аутоимуност су предиспонирајући фактори. Изостанак лактације постпартално и касније немогућност успостављања менструалног циклуса су најчешћи презентујући симптоми. Мали број болесница са Шихановим синдромом има нагли настанак тешког хипопитуитаризма непосредно постпартално, најчешће у виду хипонатремије тешког степена која се може детектовати већ у акутној фази после порођаја. Централна адренална инсуфицијенција је највероватнији узрок хипонатремије, мада је у појединим случајевима описан и синдром неадекватне секреције антидиурезног хормона.

Приказ болесника Болесница стара 39 година примљена је на Одељење ургентне интерне медицине због тешке хипонатремије праћене неуролошком симптоматологијом (*Na* 103 *mmol/L*, *Cl* 72 *mmol/L*, *K* 3,7 *mmol/L*), уз податак о изостанку лактације. Претходно је шестог постпарталног дана рехоспитализована на Клинику за гинекологију и акушерство, због тешке главобоље, мучнине, повраћања, замућења вида. Тегобе перзистирају од момента терминског вагиналног порођаја, током ког је болесница изгубила око 1000 *ml* крви због акушерских компликација. Започето је лечење применом хипертоног раствора натријума уз накнадну рестрикцију уноса течности. У оквиру дијагностикованог панхипопитуитаризма започета је супституциона терапија хидрокортизоном и потом левотироксином. Искључено је постојање инсипидног дијабетеса. Током даљег праћења, уведена је супституциона терапија хормоном раста и комбинацијом прогестерона и прогестагена.

Закључак Рана дијагноза Шихановог синдрома је од суштинског значаја. Питуитарна инсуфицијенција код ових болесница има велики диверзитет у презентацији, што понекад може да резултира комом и смрћу.

Кључне речи: постпартална хеморагија; Шиханов синдром; панхипопитуитаризам

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Isolated jejunal perforation – hidden danger in blunt abdominal trauma

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SUMMARY

Introduction Isolated jejunal perforation (IJP) without any associated injuries is rare in blunt abdominal trauma (BAT). It most commonly occurs in decelerating trauma. Diagnosis of traumatic intestinal perforation may be difficult in the first hours after injury so unrecognized "missing" intestinal injuries incidences are as high as 24%. Unrecognized traumatic bowel perforation without adequate treatment leads to the intestinal leakage into the peritoneal cavity, leading to secondary peritonitis and potentially lethal complications.

Case outline We presented the case of a 43-year-old woman injured in a road traffic accident. She was admitted to emergency surgery after diagnostic procedures according to the protocol for trauma. Initial examination, and body computed tomography (CT) revealed orthopedic injuries. Daily monitoring and follow-up examinations were done, she did not complain of any discomfort nor pain in the abdomen, and there were no signs of abdominal injury. Two days after trauma, follow-up abdominal CT revealed highly suspected jejunum perforation, still without signs of pneumoperitoneum. Laparotomy was performed and diagnosis of IJP was confirmed. Bowel perforation was surgically closed in two layers, followed by drainage of septic collections, abdominal saline lavage and primary abdominal closure. The patient was discharged on the seventh postoperative day without complications.

Conclusion In the case of BAT due to severe traumatic force in patient with nonspecific clinical signs of abdominal trauma on initial clinical and radiological examination, follow-up in a short period is necessary, to detect hidden jejunal perforation. Surgery is a life-saving for those patients and treatment of these injuries usually require simple operative procedures.

Keywords: isolated jejunal perforation; blunt abdominal trauma; computed tomography; surgery

INTRODUCTION

Isolated jejunal perforation (IJP) in blunt abdominal trauma (BAT) is severe but an extremely rare injury [1]. Although small intestine trauma is the third most common organ injury in BAT, those injuries have a low incidence of 1–5%, with bowel perforation in only 0.3% patients [1–4]. However, what is important to point out is that missing intestinal injuries have incidences as high as 24% [1–4].

In most cases, jejunal perforation is associated with injuries of solid or other hollow abdominal organs. Jejunal injury usually occurs in BAT during deceleration. This mechanism of injury in the traffic accidents is created due to a strong decelerating force between human body and seatbelt. Furthermore, the intestinal loops can be suddenly pressed between the anterior abdominal wall and the spinal column, which leads to increased pressure in the bowel lumen and perforation [3–6]. Significant blunt small bowel and mesenteric injuries (SBBMI) requires surgical treatment are: bowel transection, bowel perforation, segment devascularization, active mesenteric hemorrhage, mesenteric injury with hematoma and intestinal ischemia [4–8]. Traumatic SBBMI without adequate treatment leads to the intestinal leakage into the peritoneal cavity, making progress in secondary peritonitis and potentially lethal complication.

Unfortunately, timely detection of IJP in polytrauma patient if there is not a clinical suspicion is difficult because symptoms are initially sparse and abdominal pathology does not dominate in clinical presentation during the first hours after trauma. Therefore, daily clinical monitoring and use of diagnostic modalities (plain abdominal radiography X-ray, ultrasound, laparoscopy) are important, but the role of computed tomography (CT) in insolate jejunal injury detection is significant for early diagnosis, treatment and outcome in these patients [5–8].

We report a case of a 43-year-old woman with IJP and non-specific clinical presentation, 7 mm isolated jejunal perforation was discovered at the follow-up CT two days after trauma, she underwent surgery and recovered successfully.

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CASE REPORT

A 43-year-old female was admitted to Emergency Surgery after sustaining injuries in a road traffic accident. She was the driver, wearing seatbelt and she had front impact without airbag deployed. The patient had no previous medical or surgical history. The clinical examination revealed spontaneous breathing and hemodynamic stability, with discrete seat belt sign on abdominal wall and multiple abrasions on the left leg. Abdominal palpation showed a mild localized tenderness. Hemoglobin level was 12.5 g/dL and white blood cell count was 33,000/uL. Plain radiography of the abdomen was normal, without pneumoperitoneum. Focused assessment sonography in trauma showed no signs of free fluid or organ injury. On CT exam there was no significant thorax, abdominal or pelvic findings. Orthopedic clinical examination and radiographic examination showed left femur fracture, right tibia and patella fracture. There was no head and chest injuries. Patient was hospitalized due to severe bone fracture, and non- operative management was initially applied including intensive monitoring, rehydration, symptomatic treatment and antibiotic therapy.

On the first day of hospitalization, clinical examinations revealed persistence of very mild abdominal tenderness and there was no muscular defense or peritoneal reaction. Follow-up ultrasound has showed oedema of jejunal loops in the left hemiabdomen, with hyperechoic reaction of mesenterium, and small amount of fluid between the loops (Figure 1). Patient was hemodynamically stable and she did not complain of abdominal pain or discomfort.

On the second day of hospitalization patient began to complain on pain during abdominal examination, with positive signs of local peritoneal irritation in left abdominal quadrant. Routine daily blood analyzes discovered leukocytosis (18,000/uL). Plain X-ray showed a few air-fluid levels, no signs of pneumoperitoneum. We indicated a follow-up CT and it showed segmental thickening of jejunal wall in left hemiabdomen with small striped hypodense lesion in at the antimesenteric border with small amount of fluid, mesenterial "fat-stranding" and thickening of adjacent parietal peritoneum but without pneumoperitoneum. (Figures 2, 3, and 4) Surgery was indicated and exploratory laparotomy confirmed an IJP. We found a single jejunal perforation the size of 7 mm at antimesenteric border, about 20 cm away from the ligament of Treitz (Figure 5). Jejunal trauma was classified according to the American Association of Surgery of Trauma (AAST) as grade II injury [9]. The perforation was hidden under the omentum that covered it. Peritonitis was enclosed in the upper left quadrant of the abdomen. There were no other abdominal organs injuries. After minimal excision of bowel edges around perforation into the healthy tissue, the jejunum was closed in two layers, followed by detailed abdominal lavage and standard abdominal closure. Parenteral nutrition and antibiotics were introduced immediately in the early postoperative course. On the third postoperative day, the patient started intaking food and fluids. The abdominal ultrasound performed after surgery revealed no presence

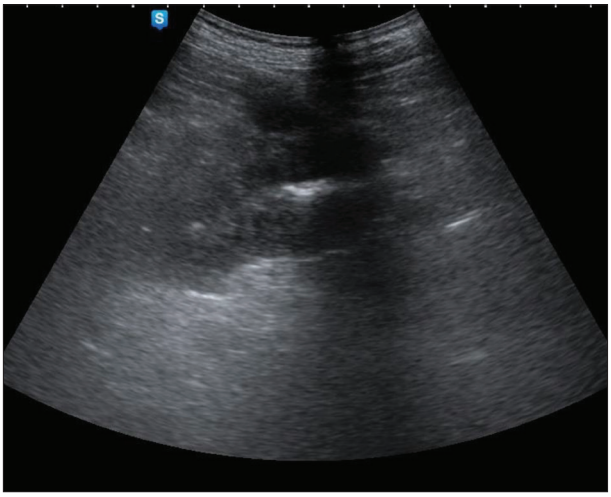

Figure 1. Transabdominal ultrasound – axial scan in the left hypochondrium: thickened and hypoechogenic jejunal wall with small amount of fluid in peritoneal cavity

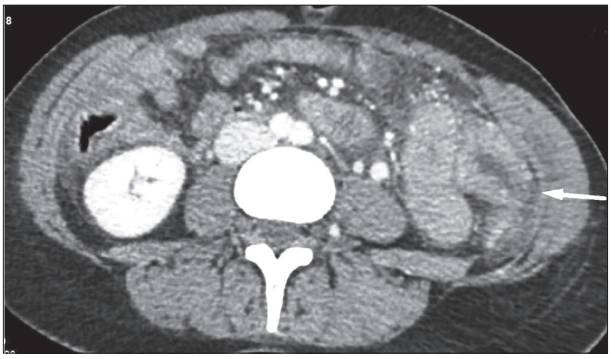

Figure 2. Contrast abdominal computed tomography – axial scan: thickened and hyperdense jejunal wall with small striped hypodense lesion at antimesenteric border (arrow) with small amount of fluid, mesenterial "fat-stranding" and thickening of adjacent parietal peritoneum – jejunal perforation

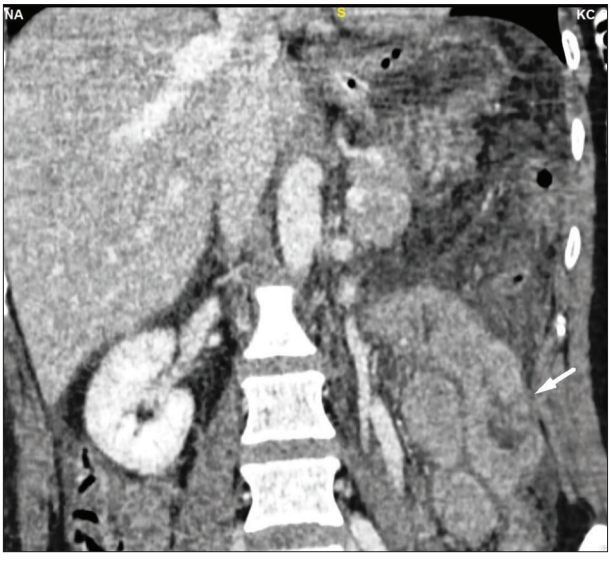

Figure 3. Contrast enhanced abdominal computed tomography – multiplanar coronal reconstruction: thickened and hyperdense jejunal wall with small striped hypodense lesion (arrow) at antimesenteric border which represent wall discontinuity – jejunal perforation

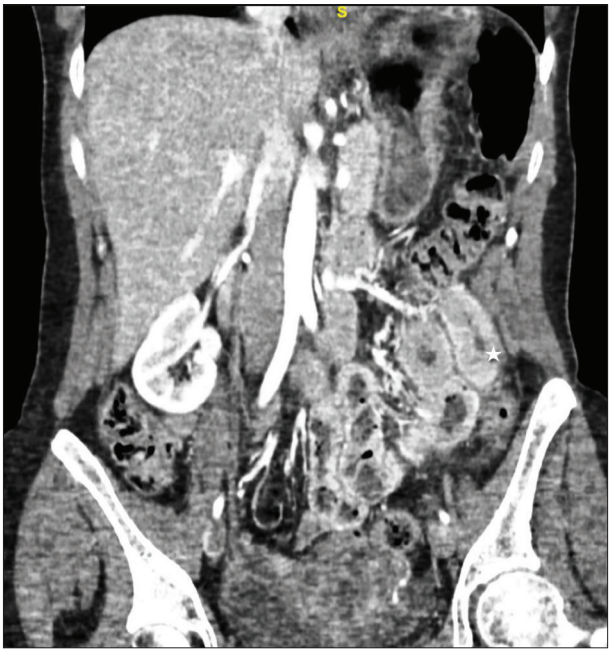

Figure 4. Contrast-enhanced abdominal computed tomography – coronal reconstruction: thickened and predominantly hyperdense jejunal wall (white star) due to traumatic injury

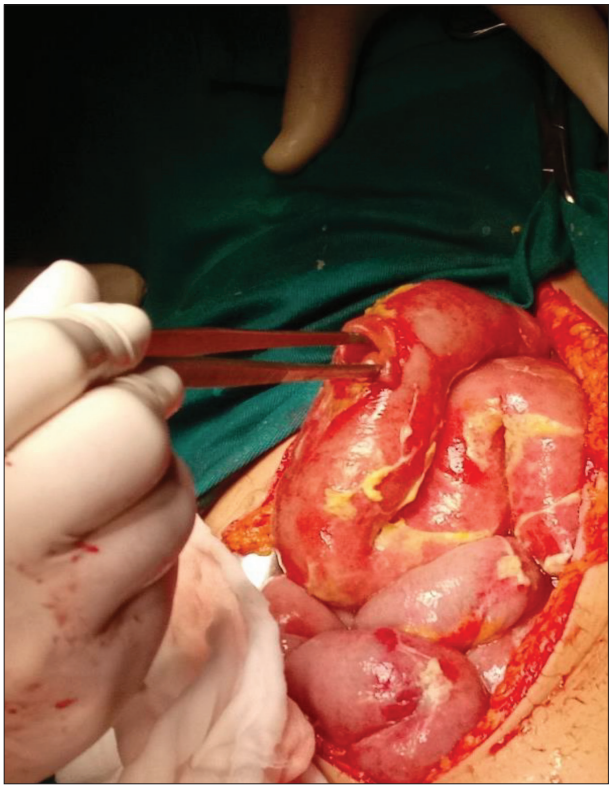

Figure 5. Interoperative finding: the single isolated jejunal perforation was found 20 cm away from the ligament of Treitz, perforation size was 7 mm at the antimesenteric border, with gaping mucosa; the isolated jejunal perforation was covered with omentum, with peritonitis enclosed in the upper left quadrant of the abdomen

of fluid or collections. Patient was discharged from the department of Emergency Surgery after seven days, without complications. She continued with orthopedic treatment and rehabilitation.

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

DISCUSSION

BAT occurs during road traffic accidents in 75% cases and it is often accompanied by serious organs injury and intra-abdominal bleeding. Small bowel injury following BAT is very rare with incidence about 1% [9, 10]. Even more rare is significant small bowel trauma with only 0.3% cases with small bowel perforation [3–6] Abdominal injury known as "seatbelt syndrome" with linear ecchymosis on abdominal wall as a result of car accident was described by Garrett and Braunstein in 1962. Simpler form of the injury called the "seatbelt sign" is expanded to the larger "seatbelt complex" which implies the existence of abdominal organs injures, thorax and spine trauma, major vascular structures trauma, ribs and sternum fractures [9, 11].

Mechanisms of small bowel perforation in BAT are clarified and basically it occurs due to the deceleration forces, bowel compression and blowout trauma due to a sudden intraluminal pressure increase in fluid-filled or air-filled bowel loops [7–10]. Diagnostic delays and unrecognized small bowel lesions in the first 24 hours, have a high incidence of 24%, despite routine use of CT [1, 2, 3]. Timely diagnosis of isolated jejunal injury can be difficult because of nonspecific clinical findings, absence of peritoneal irritation during abdominal palpation and nonradiological signs of injury immediately after trauma. We presented a case of a patient with significant small bowel injury although it was just one IJP, without signs of perforation during 24 hours after the car accident. Patient had a "seatbelt sign" on abdominal wall as the only marker of abdominal trauma.

The diagnosis of SBBMI is based on mechanism of injury, a precise follow-up clinical examination, laboratory results, radiologic methods (abdominal X-ray, ultrasound, CT), Diagnostic peritoneal lavage and laparoscopy [3–8]. Initial clinical examination can be nonspecific during first hours after the BAT. Radiological methods play an important role in the diagnosis of jejunal perforation in the absence of specific clinical symptoms. Lawson et al. [12] suggest that era "trauma scan" leads to reduction missing body injuries, but the bowel injuries are still the most commonly missed and the key to success in treatment is clinical doubt about intestinal trauma in severe trauma patients. Matsushima et al. [13] analyzed 7875 blunt trauma patients, they found blunt hollow viscus and mesenteric injury (BHVMI) in 67 (0.8%) patients who were divided in two groups: non-ischemic-BHVMI (perforation, laceration, and hematoma without devascularization) and ischemic-BHVMI (devascularization confirmed at laparotomy). Authors found that even using an advanced imaging

technique, the diagnosis of I-BHVMI can be delayed, with significant negative impact on patient outcome [12].

Based on the clinical observations and significant findings several scores have been presented to evaluate the extent of small intestine and mesenteric injury: American Association of Surgery of Trauma (AAST) and Injury Scoring Scales, Mesenteric Injury Score (MIS), Z-Score, Bowel Injury Prediction Score (BIPS) [3–6]. BIPS introduced by McNutt et al. [7] is able to predict risk of bowel injury if two or three parameters are positive on admission: high grade mesenteric injury on CT scan (presence of a mesenteric contusion or hematoma with associated bowel wall thickening and inter-loop fluid collection, or an active vascular/oral contrast extravasation, bowel transection or pneumoperitoneum), increased white blood cell (> 17,000) and abdominal tenderness. The degrees of small bowel injury classified according to the AAST are: Grade I include hematoma or laceration without perforation; Grade II is characterized by a laceration of less than 50% of the circumference; Grade III includes laceration of more than 50% of the circumference without transection; Grade IV includes transection of the small bowel; Grade V includes transection of the small bowel with loss of segmental tissue or vascular injury with segmental devascularization [7, 12].

In our patient, IJP was presented as 7 mm of a single jejunum perforation with secondary peritonitis, jejunal trauma classified as AAST grade II injury. Clinical history plays a particularly important role in determining the appropriate imaging examination for evaluating a possible perforated viscus [13].

In IJP, a plain abdominal X-ray can rarely show free air in peritoneal cavity –pneumoperitoneum, as a significant sign of hallow viscus perforation [8]. More often radiography can show indirect signs of trauma such as the presence of air-fluid levels due to paretic intestinal loop [8]. Noninvasive and simple method for abdominal examination is the ultrasound. It is sensitive in the detection of the small intestine wall edema as well as free fluid in the surrounding peritoneal space and may sometimes detect free intraperitoneal air [14]. These almost non-specific findings on ultrasound should be properly interpreted in trauma because they can lead to the diagnosis if there is clinical suspicion. CT is the most reliable method for detection of small bowel perforation (sensitivity 92%, specificity 94%) [7, 15, 16, 17]. Recent systematic reviews increasingly support performing CT scans of the abdomen and pelvis without the need for positive oral contrast in most clinical situations [18]. Small bowel perforation on CT is evident in the presence of direct signs such as bowel wall discontinuity, extraluminal air or presence of extraluminal contrast, or indirect CT signs: bowel wall thickening, abnormal bowel wall enhancement, presence of abdominal abscess formations [7, 15, 16, 17] or mesenteric fat stranding and a moderate to large volume of unexplained intraperitoneal fluid in the absence of solid organ injury [19]. CT signs that correlated with mesenteric laceration are abdominal wall injury, mesenteric contusion, free fluid in peritoneal cavity, segmental bowel hypoenhancement, and bowel hyperenhancement adjacent to a hypoenhancing segment [20]. CT scan also indicates the location of the bowel perforation. In our patient, the abdominal ultrasound, a plain X-ray and a CT scan were performed and did not show perforation of the jejunum immediately after accident. However, a follow-up CT scan showed that there was a thickening of the proximal jejunal wall that could indicate injury (Figure 4).

There is still no consensus in the literature regarding the effects of delayed surgical treatment of traumatic small intestine perforation, but it is generally accepted that morbidity and mortality is significantly lower in the case of early diagnosis and emergency surgical treatment [21, 22]. SBBMI without adequate treatment can be associated with severe complications and catastrophic outcome, because of the potential of these injuries to complicate and lead to secondary peritonitis, sepsis, and multiple organ dysfunction syndrome. Fakhry et al. [23, 24] showed on 198 patients that diagnostic delays can influence the outcome in patients with blunt small bowel injury: mortality increased from 2% for patients with delays treatment of under eight hours to 30.8% for patients treated after 24 hours.

Faria et al. [25] introduced the term "killing time." They found that all postoperative deaths in patients with both blunt and penetrating bowel injuries occurred in those who were operated after the first 24 hours.

Mingoli et al. [26] found that leukocytosis and delayed treatment (over six hours) are independent predictors of postoperative morbidity.

Therefore, in trauma patients, surgeons require a high index of clinical suspicion and early recognition which, followed by adequate treatment, is of crucial importance.

The detection of isolated jejunal trauma as the only injury in BAT is a challenge because these injuries can be clinically silent and radiologically hidden during the first hours and even days after trauma. Missing small bowel perforation with secondary peritonitis without treatment can be followed by deadly complications.

We must always suspect small intestine perforation in case of BAT. In that light intensive clinical monitoring and radiology follow-up are imperative. Surgery is life-saving for those patients and treatment of these injuries usually require simple operative procedures.

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Изолована перфорација јејунума – скривена опасност у тупој абдоминалној трауми

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САЖЕТАК

Увод Изолована перфорација јејунума без других придружених повреда је ретка у случају тупе абдоминалне трауме. Најчешће се дешава у случају децелерације. Дијагностика трауматске интестиналне перфорације може бити веома тешка у првим сатима након повреде, те је инциденца непрепознатих повреда црева 24%. Непрепозната трауматска перфорација црева без оптималног третмана доводи до цурења цревног садржаја у перитонеалну дупљу, што доводи до секундарног перитонитиса и могућег леталног исхода.

Приказ болесника Приказујемо случај жене старе 43 године која је повређена у саобраћајној несрећи. Она је примљена на одељење ургентне хирургије након спроведених дијагностичких процедура по протоколу за трауму. Иницијални преглед и компјутеризована томографија су показали ортопедске повреде. Учињени су свакодневни мониторинг и контролни прегледи, који нису показали знаке абдоминалне повреде, а болесница се није жалила на нелагодност или бол у абдомену. Два дана након повреде учињен је контролни преглед абдомена компјутеризованом томографијом, који је показао високосуспектну перфорацију јејунума, и даље без знакова пнеумоперитонеума. Учињена је лапаротомија и потврђена је дијагноза изоловане перфорације јејунума. Перфорација црева је хируршки збринута у два слоја уз дренажу инфицираних колекција, абдоминалну лаважу и трбух је потом затворен по слојевима. Болесница је отпуштена из болнице седмог постоперативног дана без компликација.

Закључак У случају тупе абдоминалне трауме услед јаке трауматске силе код болесника са неспецифичним знацима абдоминалне трауме на иницијалним клиничким и радиолошким прегледима неопходна је рана контрола ради детекције евентуалне перфорације јејунума. Хирургија је од виталног значаја за ове болеснике, а третман обично подразумева једноставне хируршке технике.

Кључне речи: изолована перфорација јејунума; тупа абдоминална траума; компјутеризована томографија; хирургија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Asymptomatic "twig-like" middle cerebral artery embryological anomaly

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SUMMARY

Introduction Anomalies of the middle cerebral artery (MCA) are very rare. "Rete MCA," "twig-like MCA" (T-MCA), "aplastic MCA," "unfused MCA," and others are all synonyms for unilateral embryological anomaly of the M1 segment of the MCA, where, due to an unknown cause, fusion of primordial arteries of the M1 segment did not happen. As a result, the M1 segment of the affected side consists of a mesh of small arteries from which arise normal perforators and cortical branches. Moyamoya disease, moyamoya-like syndrome, atherosclerotic steno-occlusive disease, vasculitis, and dissection of the MCA should be considered in differential diagnosis.

Case outline We present a 60-year-old female patient with twig-like left MCA, incidentally diagnosed due to persistent headaches six days prior to admission. Non-contrast computed tomography head examination was without peculiarities. Computed tomography angiography showed a network of small vessels in place of the left M1 segment, bridging internal carotid artery terminus with branches of the MCA bifurcation and giving rise to lenticulostriate arteries. Fourteen months later, on physical examination, the patient was in good general condition, without a neurological deficit, with occasional episodes of headache no stronger than 3–4/10 on the visual analogue scale.

Conclusion We report a patient with extremely rare variation of the M1 segment of the left MCA, incidentally diagnosed due to headaches.

Keywords: twig-like MCA; middle cerebral artery; CT angiography

INTRODUCTION

Anomalies of the middle cerebral artery (MCA) are very rare. They are less commonly seen than those of other major intracranial arteries [1, 2, 3]. Typically, three MCA anomalies (variations) are described: duplication (D-MCA), fenestration (F-MCA), and the presence of an accessory branch (A-MCA) [4]. "Rete MCA," "twig-like MCA" (T-MCA), "aplastic MCA," "unfused MCA," and others are all synonyms for unilateral embryological anomaly of the M1 segment of the MCA, where, due to an unknown cause, fusion of the primordial arteries of the M1 segment did not happen [1, 2, 3, 5, 6, 7]. As a result, the M1 segment of the affected side consists of mesh of small arteries from which arise normal perforators and cortical branches [7].

Interestingly, the variations or possible anomalies in morphology of terminal branches of the internal carotid artery, like fenestration of the anterior cerebral artery (ACA) and hypoplastic ACA, have been also described in healthy non-human primates, as well as the left/right asymmetry in morphology of the MCA [8].

CASE REPORT

A 60-year-old female patient was admitted to the Emergency Center, University Clinical Center of Serbia, due to persistent headaches six days prior to admission. Non-contrast computed tomography head examination was without peculiarities. Computed tomography angiography showed a network of small vessels in place of the left M1 segment, bridging the internal carotid artery terminus with branches of the MCA bifurcation and giving rise to lenticulostriate arteries. Left MCA M2 branches, although somewhat "paler," appeared to be normally filled with contrast agent. Deep middle cerebral vein had anomalous drainage into the left superior petrosal sinus (Figures 1 and 2).

Since this was an incidental finding, the patient was discharged home with only symptomatic therapy for headache (paracetamol/acetaminophen). Also, antiplatelet therapy in the form of acetylsalicylic acid (ASA) was prescribed. Fourteen months later, on physical examination, the patient was in good general condition, without a neurological deficit, with occasional episodes of headache no stronger than 3–4/10 on the visual analogue scale (VAS).

This case report was approved by the institutional ethics committee, and written consent

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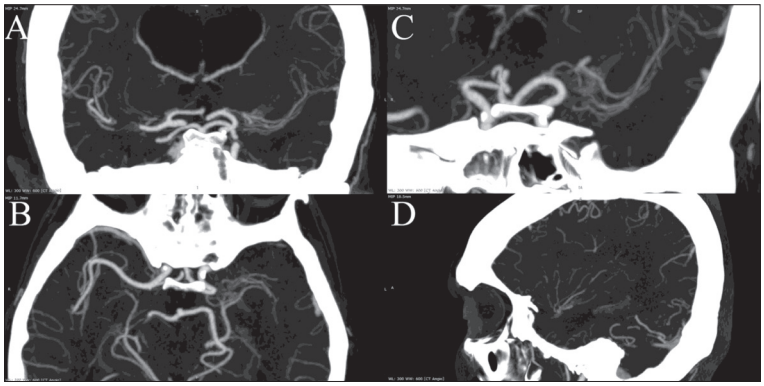

Figure 1. Computed tomography angiography maximum intensity projection reconstructions, A – coronal, B – axial, and C – oblique projections show multiple "twig-like" arteries arising from terminal part of left internal carotid artery, forming network in place where the M1 should be; D – sagittal projection demonstrates normal arborization of the left middle cerebral artery with branches that are slightly less filled with contrast agent

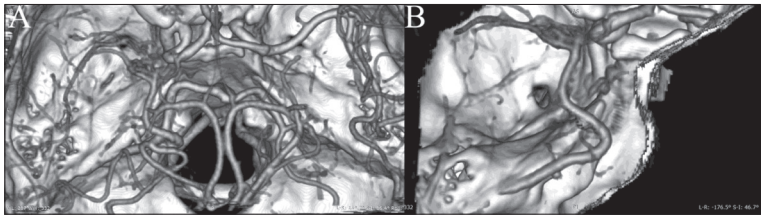

Figure 2. Computed tomography angiography volume rendering; A and B show deep middle cerebral veins anomalous drainage into superior petrosal sinus

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

DISCUSION

The MCA is the largest and most complex artery supplying the brain, vascularizing the largest territory of neocortex [9, 10]. MCA develops after ACA, when fetal plexiform network of multiple small arteries fuse and regress in order to form perforating branches of the M1 segment and the main trunk of the MCA (M1 segment). Disruption of this process, by a still unknown cause, leads to MCA developmental anomalies [7]. Fukuyama reported one case of Ap/T-MCA associated with *RNF213* mutations, which was previously believed to be associated exclusively with moyamoya [11]. In "T- MCA," this plexiform network persists unilaterally in place of the M1 segment, while cortical and perforating branches, although filled with contrast agent with discrete delay, appear to be normal [6, 7]. Of all the MCA anomalies, T-MCA is the one least commonly seen. Reports range 0.1–4% prevalence, while Viso et al. [3] reported a prevalence of 0.088% in their cohort which included over 10,000 patients.

The possibility of hypoperfusion and, eventually, ischemic events has been described [6]. Uchiyama et al. [12] reported intracerebral hemorrhage in patient two years after transient ischemic attack and diagnosed T-MCA as the culprit. Also, there is an increased risk of aneurysm formation, due to hemodynamic stress and network vessels' fragile histological architecture which can lead to rupture and hemorrhage [3, 5, 6, 13]. Sakai et al. [14] reported rupture of a *de novo* formed aneurysm arising from the twig-like network of an anomalous collateral artery associated with aplastic or twig-like MCA (Ap/T-MCA) in a patient who had ruptured aneurysm on the A1 segment four years earlier.

Moyamoya disease, moyamoya-like syndrome, atherosclerotic steno-occlusive disease, vasculitis, and dissection of the MCA should be considered in differential diagnosis [1, 3, 15].

Therapy options may vary depending on patient symptoms and angiographic findings, but no universal treatment has been established to this day [5]. If T-MCA is an asymptomatic, coincidental finding, the patient should be counseled and warned about the nature of the anomaly. Vessels in the mesh are functional but also fragile, so no intervention should be performed unless necessary [1]. It is still unclear if microsurgical superficial temporal artery bypass is beneficial in cases of recurrent ischemic events. In their case report, Matsunaga et al. [6] stated that postoperative magnetic resonance angiography showed a decrease of blood flow in aberrant network indicating that this approach may improve perfusion of affected MCA territory and lower hemodynamic stress in the aberrant network. On the other hand, Matsuo et al. [16] stated that there is no evidence that revascularization is an effective approach in preventing stroke on the affected side. Further studies on this anomaly are necessary to understand its nature and provide adequate therapy. Aneurysms in an anomalous MCA network have high risk of rupture and should be treated surgically or by endovascular embolization. Open surgery is more commonly used due to higher risk of endovascular approach through these fragile vessels [7].

Although uncommon, clinicians should recognize this vascular entity in order to avoid misdiagnosis and unnecessary treatment which can lead to catastrophic adverse events, especially in the era in which mechanical thrombectomies are becoming an everyday practice, and this entity could lead to confusion because of simulating a thromboembolic event. Less experienced neuroradiologist could easily overlook the subtle vessel network between the internal carotid artery and the distal part of the MCA.

In our opinion, the patient should be on lifelong preventive antiplatelet therapy (ASA) in order to avoid consequences of steno-occlusive and thromboembolic events. A follow-up physical examination, by a neurologist/neurosurgeon, should be performed every two years, while the neuroradiological examination is reserved only for patients with hemorrhagic or ischemic symptoms.

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Асимптоматска ембриолошка аномалија средње церебралне артерије „налик гранчици“

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САЖЕТАК

Увод Аномалије средње мождане артерије (СМА) веома су ретке. *Rete MCA*, *twig-like MCA* (*T-MCA*), „апластична СМА“, „нефузионисана СМА“ и други називи синоними су за једностарну ембриоланлу аномалију сегмента М1 СМА, где, из непознатих разлога, није дошло до фузије примордијалних артерија сегмента М1. Као резултат, сегмент М1 захваћене стране састоји се из мреже малих артерија из које полазе нормални перфоратори и кортикалне гране. Диференцијално дијагностички треба разматрати болест мојамоја, синдром сличан мојамоји, атеросклеротичну стенооклузивну болест, васкулитис и дисекцију СМА.

Приказ болесника Приказујемо жену стару 60 година са СМА „налик гранчици“, случајно откривеној због перзистентних главобоља шест дана пре пријема. Бесконтрастна компјутеризована томографија главе није показала неуобичајености. Копјутеризована томографска ангиографија приказала је мрежу малих крвних судова уместо левог сегмента М1, која је повезивала терминални сегмент унутрашње каротидне артерије са гранама бифуркације СМА и лентикулостријатним артеријама. Четрнаест месеци касније, на контролном физикалном прегледу, болесница је била доброг општег стања, без неуролошког дефицита, са повременим епизодама главобоље не јачим од 3–4/10 на визуелној аналогној скали.

Закључак Приказујемо болесницу са екстремно ретком варијацијом левог сегмента М1 СМА, случајно откривеном због упорних главобоља.

Кључне речи: СМА „налик гранчици“; средња мождана артерија; копјутеризована томографска ангиографија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

A rare case of spontaneous perirenal hemorrhage – Wunderlich syndrome

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SUMMARY
Introduction Spontaneous perirenal hemorrhage or Wunderlich syndrome represents a rare entity in urological settings. The vast majority of the causes are represented by angiomyolipoma and renal cell carcinoma. In other cases, vascular abnormalities, polycystic kidneys, polyarthritis nodosa, or pyelonephritis could represent the cause of perirenal bleeding. The treatment depends on the clinical parameters at the presentation as well as on the possible presence of renal malignancies.
Our goal was to present a rare case of a healthy men who presented the idiopathic Wunderlich syndrome.
Case outline We present a case of a 50-year-old patient with spontaneous perirenal hemorrhage that was not due to any defined cause even after a six-months follow-up.
Conclusion In some rare cases of perirenal bleeding, the cause cannot be defined at the presentation, even with today's advanced radiological imaging methods. Thus, it is important to be aware of the fact that in those cases, a longer follow-up is needed, knowing that the presence of perirenal hematoma can always obscure the real diagnosis. Sometimes, even in cases where the proper follow-up has been done, the real cause of the bleeding remains unknown.
Keywords: Wunderlich syndrome; perirenal hematoma; angiomyolipoma

INTRODUCTION

Wunderlich [1] was the first who presented the spontaneous hematoma of the kidney in 1856. It refers to spontaneous non-traumatic renal bleeding into subcapsular and/or perirenal space. This condition may be caused by various pathologies, such as benign and malignant renal tumors, renal artery aneurysms, polyarteritis nodosa, polycystic kidneys, renal infections, or undiagnosed hematological conditions [2]. Some of the possible causes with their respective percentages are given in Table 1 [2, 3].

Table 1. Possible causes of Wunderlich's syndrome in percentage

Wunderlich's syndrome causes	%
AML	23
RCC	19
ACKD	8
Simple renal cyst	8
Sarcoma	4
Hematoma or hemorrhage only	38

AML – angiomyolipoma; RCC – renal cell carcinoma; ACKD – acquired cystic kidney disease

The treatment depends mainly on patient conditions and the determination of the cause of the hemorrhage. Since misdiagnosis is an emerging topic in modern medicine and there are disciplines that confirm an increasing alert on the risks of an omitted diagnosis or the consequences of incorrect treatment, we consider it important to present a rare case of spontaneous perirenal hematoma which was not due to any known or diagnosed cause [4, 5].

CASE REPORT

A 50-year-old male patient was admitted to the emergency room with acute abdominal and right flank pain and painful sensitivity to palpation. The patient did not report any history of trauma or drug use and the anamnesis did not reveal other illnesses. During clinical examination, the blood pressure of 120/80 mmHg and the heart rate of 82 beats per minute were recorded. The hemoglobin value was 13.8 g/dl, leukocytes 15,000/mm^3, platelet count amounted to 270 × 10^9/L, creatinine clearance was 0.88 mg/dl, coagulation parameters as prothrombin time, activated partial thromboplastin time, and international normalized ratio were in their respective normal ranges. Urine sediment showed only proteinuria. Ultrasound examination followed by computed tomography (CT) scan of the abdomen showed a very large right perirenal hematoma without showing a mass responsible for the hemorrhage. The patient was symptomatic and the flank pain was at that moment not very responsive to conservative therapy. An urgent arteriography was performed showing no acute vascular bleeding sites so that there was no need for arterial embolization. The patient was treated with intravenous antibiotic therapy and again with intravenous pain medications, this time with success. On the next day hemoglobin values decreased from 13.8 g/dl to 8.8 g/dl, so that blood transfusion was needed. Two units of blood were transfused so that the hemoglobin values increased and remained stable (11 g/dl)

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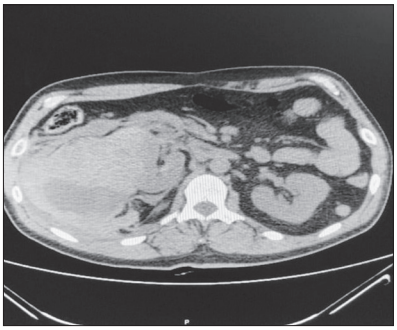

Figure 1. Abdominal computed tomography scan of the right retroperitoneal bleeding at the presentation

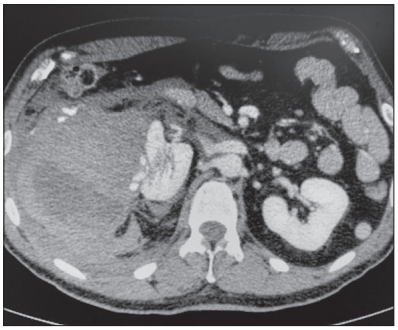

Figure 2. Abdominal computed tomography scan two days after the acute event

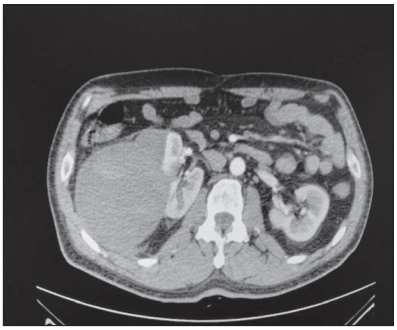

Figure 3. Computed tomography angiogram one month after the acute event

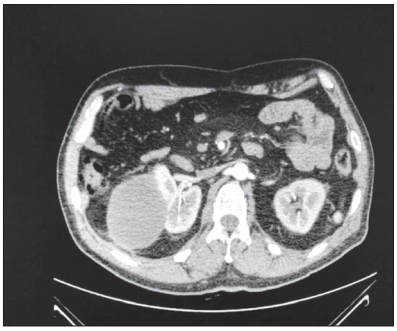

Figure 4. Computed tomography angiogram three months after the acute bleeding

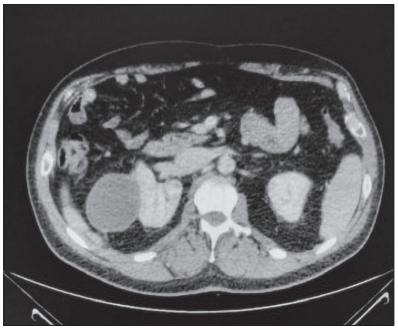

Figure 5. Computed tomography angiogram at six months follow-up

one day later. Two days after the acute event, the control CT scan showed a small increase of subcapsular hematoma, still without any sign of acute bleeding. After the close follow-up, the patient was dismissed asymptomatic and with stable hemoglobin levels on day 8 after the acute episode.

The control angio-CT scan after one, three, and six months from the acute event showed the important reduction of the perirenal hematoma without apparent cause for the previous bleeding.

Figures 1–5 show the CT scan of the right perirenal hematoma from the first presentation to the six-month follow-up: Figure 1 shows the bleeding at the presentation, Figure 2 shows CT scan two days after the acute event, Figure 3 shows the control angio-CT scan at 1 month after the acute event, Figure 4 shows the control angio-CT scan at three months after the acute bleeding and Figure 5 shows the control angio-CT scan at six months' follow-up.

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

DISCUSSION

Spontaneous renal bleeding to the subcapsular and perinephric space, known as Wunderlich syndrome (WS), could occur due to benign or malignant renal tumors, vascular lesions such as polyarteritis nodosa, anatomical lesions as renal cysts, renal infections, or nephritis [2]. Between 2003 and 2011 just 250 cases have been reported [6, 7]. Renal neoplasms are the most common cause of WS, accounting for 60–65% of all cases in which renal angiomyolipoma is the most common benign neoplasm responsible for WS [5, 7]. Renal cell carcinoma could also be the cause of WS, but in only 0.3–1.4% of cases [7]. This potentially life-threatening condition which could be associated with hypovolemic shock is also followed by other symptoms, such as acute lumboabdominal pain and palpable abdominal mass, symptoms forming the so-called Lenk's triad [2]. Although clinical guidelines for managing WS are not yet well established, the appropriate treatment for patients with WS depends on the right diagnosis that a bleeding has occurred, in which contrast-enhanced CT scan, as a method of choice with a sensitivity of 100%, has the most important role [7]. Although successful in the diagnosis of perirenal hemorrhage, CT scan has much lower sensitivity in identifying the renal neoplasm causing hemorrhage [8, 9].

If initial CT scan shows no mass responsible for the bleeding, angiography should be performed in order to reveal eventual vascular lesions and to perform embolization [10]. This could be very helpful especially in some rare cases such as segmental arterial mediolysis, as reported by Skeik et al. [11].

Thus, angiography and arterial embolization represent an important tool in diagnosis and therapy of spontaneous retroperitoneal hemorrhage. In some cases, as in the case of spontaneous retroperitoneal bleeding due to metastatic testicular germ tumor, as reported by Luis Eduardo et al. [12], it is not possible to dominate the bleeding just by using embolization. In those cases, an exploratory laparotomy with or without partial nephrectomy is needed [12]. A surgical intervention is necessary every time the hypovolemic shock caused by acute bleeding cannot be dominated by arterial embolization.

Thus, the main problem in WS is defining the source of bleeding in order to postulate the correct therapy.

In our case, it was not possible, in the acute phase, to define the cause of bleeding. Fortunately, the bleeding limited itself spontaneously and the patient was treated conservatively. This did not ensure that the bleeding source was not still present. The literature reports that if a CT scan, followed by angiography, does not reveal the bleeding source, a CT scan should be repeated later, as it is obvious that if the hemorrhage is massive, a possible renal cell carcinoma, angiomyolipoma, or other renal bleeding sites, such as renal cysts, could be seen just after the resorption of the hematoma [13]. That has also been proven, not just in cases of renal angiomyolipoma or clear cell carcinoma, but also in rare cases of renal sarcoma presenting WS [14]. Thus, we performed a CT scan at one, three, and six months after the acute phase. As seen from the presented figures, we were not able to elucidate the real cause of bleeding even six months after the acute event.

It is worth mentioning that hematologic issues can contribute to WS. As reported, some patients with end-stage renal disease are predisposed to bleeding diathesis in the setting of uremic platelet dysfunction, anemia, irregularities in von Willebrand factor, and impaired platelet – vessel wall interaction [15, 16].

All those factors were excluded in our case, given the young age, complete negative anamnesis and normal laboratory findings of the patient.

The patient was treated conservatively, which corresponds to previous findings on WS, stating that if the patient is hemodynamically stable in the acute phase, nephrectomy or partial nephrectomy should be deferred. A recent Korean study of 28 patients with WS stated that the definitive treatment of WS will depend on the clinical condition and the underlying cause, with possible therapeutic options including conservative therapy, angioembolization, nephron-sparing surgery, or radical nephrectomy [3, 17]. More interestingly, they found that five of 28 patients had no obvious cause of perirenal bleeding. This was also the case with our patient, given that nephrectomy or partial nephrectomy were not needed even later, as no malignant pathology could be observed.

In conclusion we can say that, although a vast majority of WS cases are represented by angiomyolipoma or by renal cell carcinoma, the cause sometimes remains unknown. In the present report we described a rare case of idiopathic WS whose cause could not be diagnosed even after six months of follow-up.

Conflict of interest: None declared.

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Редак случај спонтане перирenалне хеморагије – Вундерлихов синдром

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САЖЕТАК

Увод Спонтана перирenална хеморагија или Вундерлихов синдром представља редак ентитет у урологији. У највећем броју случајева узрок овог феномена представљају ангиомиолипоми и карциноми бубрега. Мање заступљени су узроци васкуларне природе, полицистични бубрези, нодозни полиартритис, пијелонефритис или идиопатски фактори. Третман ових болесника зависи од клиничких параметара на пријему, као и од присуства евентуалних малигних бубрежних обољења.

Наш циљ је био представити редак случај релативно младог болесника са Вундерлиховим синдромом који није био узрокован ниједном до данас знаних патологија.

Приказ болесника Приказујемо случај 50-годишњег болесника са спонтаним перирenалним крварењем чији узрок није откривен ни после шест месеци праћења од акутног крварења.

Закључак У случајевима перирenалног крварења, узрок не може увек бити откривен по пријему болесника, упркос данашњим врло развијеним радиолошким методама. У овим случајевима важно је имати на уму да је дуже праћење ових болесника, после акутног крварења, од суштинске важности за успостављање праве дијагнозе, с обзиром на то да перирenални хематом може маскирати присуство евентуалног малигнитета. У врло ретким случајевима узрок перирenалног крварења не може бити откривен ни после дужег праћења.

Кључне речи: Вундерлихов синдром; перирenална хеморагија; ангиомиолипом

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Prognostic value of optical coherence tomography in chronic chiasmal compression

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SUMMARY

Introduction Sellar and parasellar region lesions, such as pituitary adenoma, often lead to the compression of the optic chiasm. Consequentialy, visual field (VF) defects and loss of visual acuity are common complaints in these patients. The aim of this report is to evaluate if optical coherence tomography, measuring retinal nerve fibre layer (RNFL) and ganglion cell complex thickness (GCC), offers a reliable prediction of visual outcome in patients with chronic chiasmal compression from a pituitary macroadenoma.

Case outline We present a case of chronic chiasmal compression from a pituitary macroadenoma with an initial binocular VF defect and low values of optical coherence tomography parameters binocularly. The average value of RNFL on the right eye pre/postoperatively was 48/79 μm, while on the left eye it was 56/63 μm. The average value of GCC pre/postoperatively was 47/46 microns on the right and 45/46 microns on the left eye. Six weeks after surgical optochiasmal decompression, macular GCC on both eyes and RNFL on the left eye remained largely unchanged, while RNFL of the right eye exhibited increases in thickness, as the postoperative consequence of the removal of the conduction block. Neither VF nor visual acuity showed postoperative improvement.

Conclusion Irreversible damage to the GCC and RNFL by longstanding compression results in poor visual outcome after surgery. Ganglion cell layer of the macula is a more accurate and reliable indicator of postoperative visual outcome.

Keywords: optical coherence tomography; macular ganglion cell layer; peripapillary retinal nerve fiber layer; visual outcome; suprasellar mass

INTRODUCTION

Compressive optical neuropathies are among the most important anterior optical pathways diseases that can lead to severe impairment of visual function. Compressive optic neuropathy is a group of diseases caused by mechanical comperssion of retinal ganglion cell (RGC) axons of the optic nerve. Chiasmal lesions may be caused by pituitary adenoma, craniopharyngioma, meningioma, cysts, and aneurysm.

Surgical removal of the lesions is an important aspect of clinical management. One of the primary indications for surgical management of chiasmal compression is the progressive loss of visual function. Surgical treatment enables decompression of the optochiasmatic complex, prevents further visual function deterioration, and enables visual acuity (VA) improvement at the same time. Visual recovery after surgical tretment of the chiasmal compression occurs in stages, with the removal of the conduction block, followed by secondary remyelination and restoration of the axoplasmic flow over months to years [1].

Pituitary adenoma is the most common anterior optical pathways' disease. As a consequence, visual impairment, including visual field (VF) defects and loss of VA, is a common complaint [2, 3].

Several predictors for the improvement of visual function after decompression of the anterior visual pathway have been studied, including duration of symptoms, age, preoperative VA, tumor size, optic disc pallor, funduscopic appearance of the retinal nerve fiber layer (RNFL), with coflicing results [3–6].

With the development of optical coherence tomography (OCT), more objective measurements of optic nerve damage and more objective prediction of visual outcome after treatment of pituitary adenomas have become available [7–19].

The aim of this report is to evaluate if OCT offers a reliable prediction of visual outcome in a case of chronic chiasmal compression from a pituitary macroadenoma. We used objective parameters of the thickness of the RNFL and the thickness of the ganglion cell complex (GCC).

CASE REPORT

A 65-year-old woman presented with an eight-month-long history of malaise, weakness, frontal headaches, and blurred vision in both of her eyes. Complete neuro-ophthalmic examination, including the VA test (Snellen charts), color vision test, VF analysis (Humphrey field analyzer;

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Carl Zeiss Meditec Inc., Dublin, CA, USA), full field 120 point suprathreshold test, ocular motility test, dilated stereoscopic fundus examination, and OCT measurements of the RNFL and the macular ganglion cell-inner plexiform layer (GCIPL) thickness, was done.

OCT imaging was conducted after pupil dilation (administration of 1% tropicamide eye drops), using the Cirrus OCT (OCT-3, OCT software version 6.0; Carl Zeiss Meditec Inc., Dublin, CA, USA). RNFL Optic Disc Cube 200 × 200 and Macular Cube 512 × 128 scan protocols were used. The ganglion cell analysis algorithm was used to determine macular GCIPL thickness within the 14.13 mm^2 elliptical annulus area centred on the fovea. Six sectoral (superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal) GCIPL thickness values were used for analysis. The Cirrus SD-OCT algorithm calculated the peripapillary RNFL thickness at each point on the circle of 3.14 mm^2 centered on the optic disc. Four-quadrant (superior, nasal, inferior, and temporal) RNFL thicknesses were used for analysis.

The patient had normal ocular position and motility with pupils of equal sizes. Dilated fundus examination revealed atrophic optic nerve head in the right eye and subatrophic optic nerve head in the left eye.

On examination, the patiet's VA (Snellen) was 0.03 in the right eye and 0.6 in the left eye, and there was a mild right relative afferent pupillary defect and red desaturation in the right eye.

VF testing demonstrated preservation of the central 30° in the nasal half of the left VF and total VF loss in the right eye.

Due to the concern of a chiasmal lesion, magnetic resonance imaging of the endocranium was performed and revealed a pituitary macroadenoma measuring 28 × 37 × 36 mm. The tumour extended supra, para, and infrasellary and throughout both cavernous sinuses, with pronounced compressive effect on the prechiasmal part of both optic nerves and the chiasma itself (Figure 1).

Additionally, there were multiple endocrinological disorders observed, including dropout of thyroid, adrenocorticotropic, somatotropic, and gonadotropic function. Patohystologic examination confirmed the case of gonadotropic adenoma, a neuroendocrine hypophyseal tumour.

Neurosurgical treatment involved subtotal tumor resection.

OCT showed pronounced thinning of RNFL (Table 1, Figure 1) and macular GCC binocularly (Table 2, Figure 4).

Nuclear magnetic resonance examination six weeks after surgical treatment revealed a larger residual lesion in the right sellar region and within the right cavernous sinus, with minimal growth of the tumor inside the left cavernous sinus. VA also stayed

Table 1. Thickness of the retinal nerve fiber layer [µm]

Parameter	Preoperative		Postoperative	
	right eye	left eye	right eye	left eye
Average thickness	48	55	79	53
Superior quadrant	50	65	66	64
Inferior quadrant	47	65	73	63
Nasal quadrant	51	48	117	44
Temporal quadrant	44	42	60	42

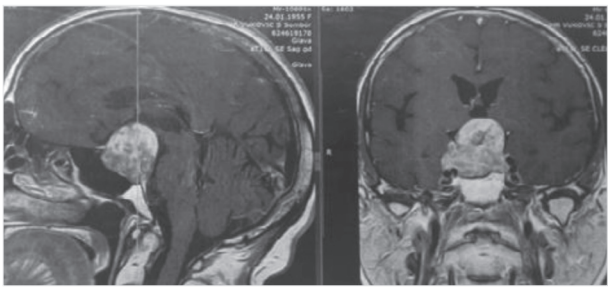

Figure 1. Nuclear magnetic resonance scan of the endocranium with optochyasmal compression

Table 2. Thickness of the macular ganglion cell layer [µm]

Parameter	Preoperative		Postoperative	
	right eye	left eye	right eye	left eye
Average thickness	47	45	46	46
Superior sector	49	44	47	44
Inferior sector	47	44	48	41
Superonasal sector	46	42	45	47
Inferonasal sector	48	40	44	42
Superotemporal sector	42	50	44	49
Inferotemporal sector	45	52	45	52

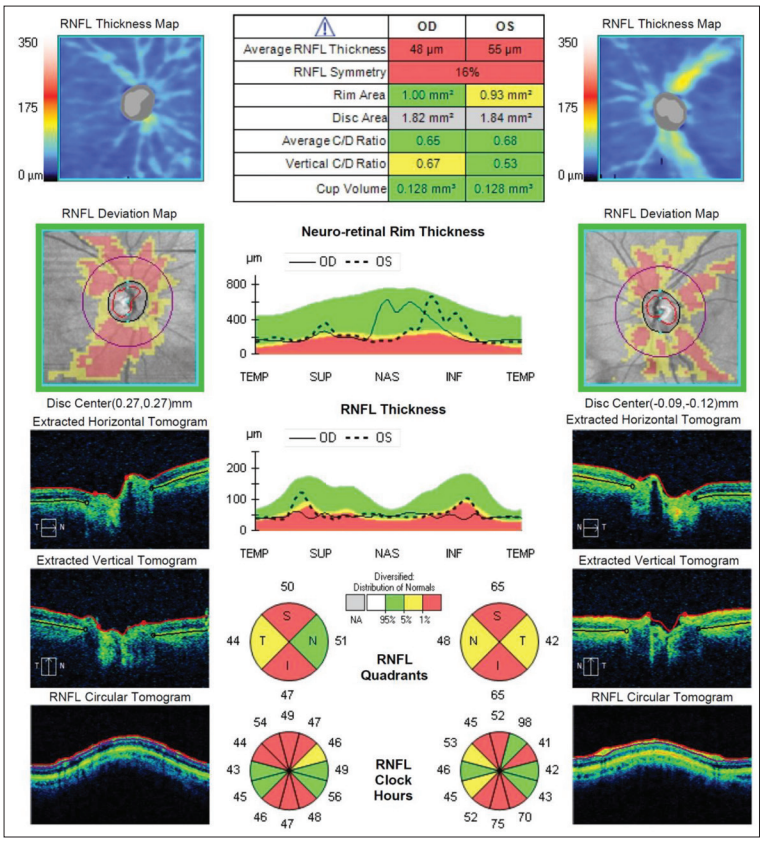

Figure 2. Preoperative retinal nerve fibre layer (RTNL) thickness

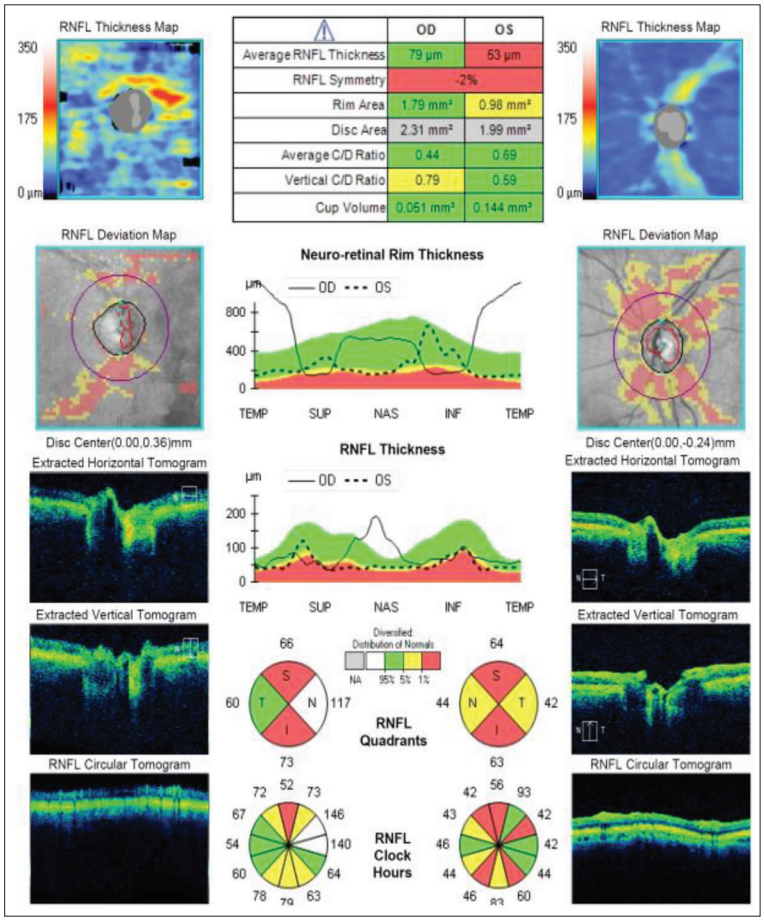

Figure 3. Postoperative retinal nerve fibre layer (RNFL) thickness

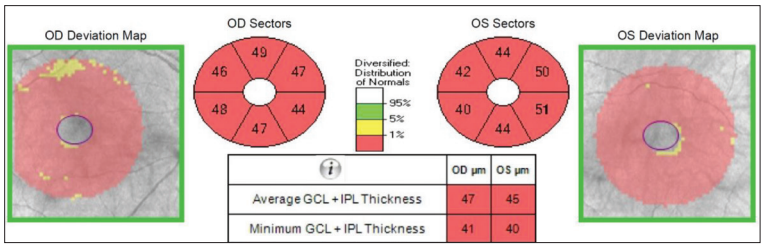

Figure 4. Preoperative ganglion cell complex thickness

GCL – ganglion cell layer; IPL – inner plexiform layer

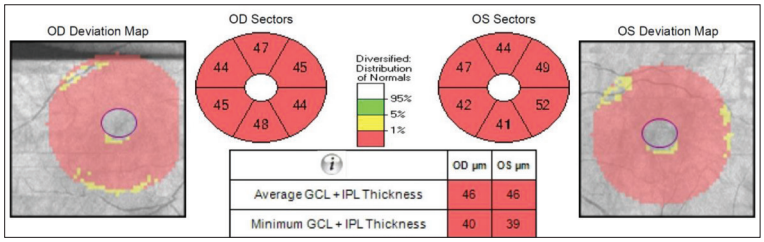

Figure 5. Postoperative ganglion cell complex thickness

GCL – ganglion cell layer; IPL – inner plexiform layer

unchanged. OCT parameters – macular GCC on both eyes and RNFL thickness of the left eye remained largely decreased, as on initial presentation, while RNFL showed signs of improvement as the consequence of postoperative removal of the conduction block (Figures 3 and 5). The VF defect was unchanged binocularly (Figures 6 and 7).

All procedures performed in this report were in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written consent to analyze and publish all shown material was obtained from the patient and the approval for the study was given by Ethics Committee of the Eye Clinic, Clinical Centre of Vojvodina.

DISCUSSION

Tumors of the sellar, suprasellar, and parasellar region, which compose 30% of all intracranial tumors according to multiple authors, are a complex neurosurgical problem even today. This is mainly the consequence of their close anatomical relations with the vital structures of this region – the internal carotid artery and its branches, the hypothalamus, the infundibulum and the pituitary gland, with the optic nerves and their chiasma.

Individual variations of the chiasmal position and the inclination of its oblique plain determine the duration of the "quiet stage" of the growth of pituitary adenoma needed for the deterioration of the visual function. The gradual, slow decline of the visual function, headaches, a mild endocrine disorder result in the late physician involvement, with already enlarged tumors of uncertain prognosis for visual recovery.

In recent years, it has been established that patients who have an objectively measurable RNFL loss and the loss of retinal GCC at the time of surgery for chiasmal compressive lesions are less likely to have recovery of VA or VF after surgery [9–16]. Thinner preoperative RNFL and macular GCC thickness were found to be associated with poorer VA and VF after surgery. This also supports the notion that preserved OCT RNFL and macular GCC thickness confer a good visual outcome.

In this case, chronic chiasmal compression caused not only a conduction block but also a significant atrophy of the RGC, confirmed with OCT parameters that remained mostly decreased.

Although our study's follow up period was only eight weeks, the results proved to be comparable with the findings of Danesh–Meyer et al. [11], which, in a series of 40 cases with chiasmatic compressive lesions, with OCT and VF analysis, showed that pre- and post-decompression treatment in patients with thin RNFL did not demonstrate significant improvement in VA and VF. Min et al. [5], Zhang et al. [15], as well as Lee

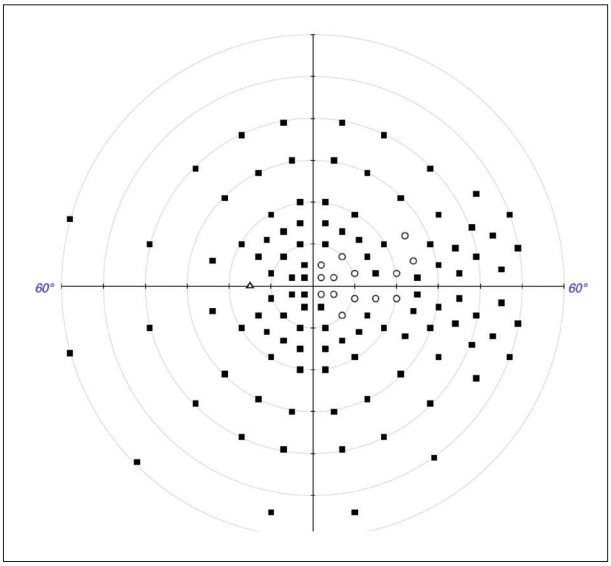

Figure 6. Full-field 120 point perimetry test of the left eye preoperatively

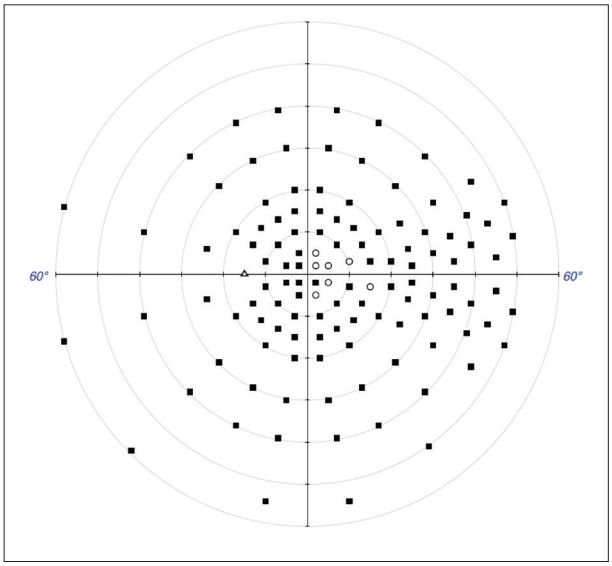

Figure 7. Full-field 120 point perimetry test of the left eye postoperatively

et al. [16] found with preoperative and postoperative RNFL thickness analysis that eyes with visual defects but normal preoperative RNFL thickness showed a significantly greater improvement in postoperative visual function than those with thin preoperative RNFL thickness. Similarly, Jacob et al. [6] demonstrated that circumpapillary RNFL thinning measured by OCT decreased the patient's chances of recovery of initial VF defect three months after treatment.

Some researchers also explored the predictive value of RNFL thicknesses in different quadrants [2, 6, 15, 17, 20]. Chiasmal compression is well-known to cause more thinning of the nasal and temporal sectors of the peripapillary RNFL thickness, and predominantly nasal hemiretina thinning of macular GCC, something we were not able to confirm in our patient due to extreme thinning of RNFL and GCL in all sectors [2, 17, 20].

While the majority of the research has focused on measuring the peripapillary RNFL, recent data suggest the ganglion cell layer – inner plexiform layer of the macula may be a more accurate and reliable biomarker of vision [6, 7, 8, 10, 12, 15, 17, 18]. According to numerous authors, GCC thinning, found in our patient as well, remaines relatively unchanged before and after decompression [17–20]. Consequently, patients with GCC loss before decompression had decreased chances of recovery of postoperative VF, the fact we can agree based on the postoperative VF in our patient [17–20].

RNFL and GCC thickness measured by OCT have been identified as useful prognostic indicators in the preoperative assessment of chiasmal compression and became an important aspect of the pre-treatment evaluation of pituitary tumors. OCT analysis may be an objective method to diagnose and follow patients with chiasmal lesions.

In the patient from our report, chronic chiasmal compression led to pronounced axonal damage, manifested in significant RNFL and GCC thinning and poor postoperative recovery of visual function. Ganglion cell layer of the macula proved to be a more accurate and reliable indicator of postoperative visual outcome.

Conflict of interest: None declared.

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Прогностичка вредност оптичке кохерентне томографије код хроничне хијазмалне компресије

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САЖЕТАК

Увод Супраселарне експанзивне лезије, као што су макроаденоми хипофизе, притиском на оптичку хијазму доводе до пада видне оштрине и испада у видном пољу најчешће на оба ока. Данас се употребом оптичке кохерентне томографије могу утврдити степен оштећења и могућност постоперативног побољшања видне функције.

Циљ рада је да се кроз приказ болесника са макроаденомом хипофизе и хроничном компресијом оптичке хијазме испита да ли мерењем дебљине слоја нервних влакана ретине и макуларног слоја ганглијских ћелија оптичком кохерентном томографијом добијамо објективну и реалну процену постоперативног стања видне функције.

Приказ болесника Приказали смо болесника са макроаденомом хипофизе и хроничном компресијом оптичке хијазме са иницијалним бинокуларним испадом видног поља, падом видне оштрине и веома ниским вредностима дебљине слоја нервних влакана ретине и макуларног слоја ганглијских ћелија на оба ока. Средња вредност дебљине слоја нервних влакана ретине преоперативно/постопертивно на десном оку износила је 48/79 микрона, а на левом 56/6 микрона. Средња вредност дебљине макуларног слоја ганглијских ћелија преоперативно/постоперативно била је на десном оку 47/46, а на левом 45/46 микрона. Видно поље на оба ока не показује постоперативно побољшање, као ни видна оштрина.

Закључак У овом случају изражено оштећење ганглијских ћелија макуле и нервних влакана оптичког нерва услед хроничне компресије потврђено је параметрима оптичке кохерентне томографије – слојем нервних влакана ретине и макуларним слојем ганглијских ћелија. Дебљина макуларног слоја ганглијских ћелија у односу на дебљину слоја нервних влакана ретине је бољи показатељ могућности постоперативног побољшања видне функције.

Кључне речи: оптичка кохерентна томографија; слој ганглијских ћелија макуле; слој ретиналних нервних влакана; исход видне функције; супраселарни тумор

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Leber's hereditary optic neuropathy with complete visual recovery – the first report

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SUMMARY

Introduction Leber's hereditary optic neuropathy (LHON) typically affects young adults, with a higher prevalence in men, but can ultimately occur at any age, as well as in women. LHON is caused by point mutations in the mitochondrial DNA. Classically, LHON presents as a subacute unilateral loss of visual acuity, dyschromatopsia in the red–green axis and a central or centrocecal scotoma. The contralateral eye usually develops similar symptoms within 3–6 months of the disease onset.

Case outline A 55-year-old male patient presented to a neurologist 20 days after the onset of vision loss. The patient was admitted as an emergency case to the Clinic for Eye Diseases due to a sudden vision loss in both eyes. The best corrected visual acuity in both eyes was 4/60. The intraocular pressure on both eyes was normal. Oedema of the optic nerve head was found on the right eye and a disc with blurred borders was seen on the left eye. During hospitalization, several consultative examinations and diagnostic procedures were performed, together with blood laboratory and visual field perimetry. Genetic testing for LHON as well as antibodies to AQ4, immunoserology, virology, and lumbar puncture were performed, as well as the visual evoked potential and ultrasound examinations.

Conclusion In our patient, the presence of a heteroplasmic mutation m.11778 G>A (*MT-ND4*) in the mitochondrial DNA analyzed from a peripheral blood sample was shown. In the available literature, this is the first documented LHON case demonstrating complete restitution of visual acuity in both eyes.

Keywords: Leber's hereditary optic neuropathy; sudden loss of vision; mitochondrial DNA mutation

INTRODUCTION

Leber's hereditary optic neuropathy (LHON) typically affects young adults with a higher prevalence in men, but it can ultimately occur at any age and in women. LHON is caused by point mutations in the mitochondrial DNA, which lead to a defect in complex I of the mitochondrial respiratory chain. This in turn causes dysfunction and later degeneration of retinal ganglion cells, followed by ascending optic atrophy [1]. Mitochondrial deficiency of respiratory complex 1 compromises adenosine triphosphate production and oxidative stress management in retinal ganglion cells. The most common LHON-causing mutations are 11778G>A, 3460G>A, and 14484T>C point mutations in *MT-ND4*, *MT-ND1*, and *MT-ND6* [2]. Classically, LHON presents as a subacute unilateral loss of visual acuity, dyschromatopsia in the red–green axis, and a central or centrocecal scotoma. The contralateral eye usually develops similar symptoms within 3–6 months of the disease onset. In 25% of the cases, however, the disease begins bilaterally [1]. Most patients deteriorate to acuities poorer than 20/200 (0.1). Pupillary light responses may be relatively preserved when compared with the responses in patients with optic neuropathies from other causes [3]. The classic fundus appearance triad includes the following: 1) hyperemia and elevation of the optic disc, with thickening of the peripapillary retina; although the disc appears swollen, it does not leak on fluorescein angiography; 2) peripapillary telangiectasia, and 3) tortuosity of the medium-sized retinal arterioles. These findings can develop before vision loss begins.

The fundus can also appear entirely normal (in > 40% of cases in one referral series) [4]. No treatment has been demonstrated to be effective. Corticosteroids are still one of possibilities for LHON treatment despite newer drugs such as idebenone or gene therapy. Idebenone may increase mitochondrial energy production and may improve the outcome of LHON. Novel therapies such as estrogen and gene therapy are being explored. Controversy exists whether tobacco or excessive alcohol intake, which might stress mitochondrial function, play an initiating role in LHON [5].

CASE REPORT

A 55-year-old male patient presented to a neurologist 20 days after the onset of vision loss. Simultaneously, he was treated by an otorhinolaryngologist for antibiotic-treated sinusitis. The patient had been treated for arterial hypertension for several years, and had been wearing a hearing aid. On examination, the neurological status was otherwise unremarkable.

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Multi-slice computer tomography of the endocranium revealed no pathology. Blood laboratory analyses were performed. Complete blood count showed mild erythropenia (4.34×10^{12}/l); mean corpuscular volume was 104 fL, and mean corpuscular hemoglobin of 35 pg. Biochemical analyses showed a high value of total bilirubin (43 μmol/L); ferritin was 325 ng/mL, aspartate transaminase was 81 U/L, alanine transaminase 145 U/L, gamma-glutamyl transferase 536 U/L. Electrolyte and inflammatory factor values were within the reference ranges.

The patient was emergently admitted to the Clinic for Eye Diseases due to a sudden vision loss in both eyes. The best corrected visual acuity in both eyes was 4/60. The intraocular pressure was 16 mmHg on both eyes measured by applanation tonometry. Signs of dry eye were found on the anterior segment examination. The optic nerve head manifested oedema on the right eye and blurry borders on the left eye.

Computerized perimetry was performed on the first, fourth, and eighth day. A centrocecal scotoma was observed in both eyes, enlarging on each subsequent image. On the first day, visual field demonstrated a mean deviation (MD) of -9.51 dB on the right eye (RE) and of -13.38 dB on the left eye (LE). On the fourth day, the MD in the RE was -9.58 dB, and in the LE it was -19.42 dB. On day 8, MD was -19.24 dB in the RE and -26.65 dB in the LE.

During hospitalization, several ancillary examinations and diagnostic procedures were performed. X-rays of the lungs and heart as well as paranasal sinuses did not show pathological changes. On the second day of hospitalization, magnetic resonance imaging of the endocranium was performed, which showed supratentorially bilateral chronic microangiopathic changes in the white matter of the brain and initial periventricular ischemic leukoencephalopathy. Chronic mastoiditis was found on both sides. Antinuclear antibodies were not detectable.

The patient was examined by an internal medicine specialist. A gastric volvulus was found and he did not receive consent for the use of pulse corticosteroid therapy at that moment. Multidetector row computed tomography of the thorax was without pathological changes.

On the third day of hospitalization, a consultant neurologist introduced pulse corticosteroid therapy. Genetic testing for LHON, as well as antibodies to AQ4, immunoserology, virology, and lumbar puncture were performed. It was advised to continue with corticosteroid treatment at the Clinic for Neurology Diseases. Visual evoked potentials testing was performed, and prolonged P100 latency was found on both eyes (right: 136 ms; left: 146 ms).

Immunoelectrophoresis was performed and it identified parallel oligoclonal IgG bands in the cerebrospinal fluid and serum with identical number and intensity. The findings support systemic immune activation. A color Doppler scan of the blood vessels of the neck indicated a moderately thickened intimomedial complex with no plaques. Carotid and vertebral arteries of regular diameter and direction were found. Transcranial color Doppler showed normal findings on the anterior and vertebrobasilar blood flow. Retroorbital ultrasound revealed regular hemodynamic parameters in central retinal and ophthalmic arteries bilaterally. Clinical decision support of the temporal artery neat was with no signs of temporal arteritis.

Detection of these mutations was performed using capillary electrophoresis on an automatic sequencer (3500 Genetic Analyzer; Thermo Fisher Scientific, Waltham, MA, USA). The results were analyzed using Sequencing Analysis Software v. 5.3.1. (Thermo Fisher Scientific). In our patient, the presence of a heteroplasmic mutation m.11778 G>A (*MT-ND4*) in mitochondrial DNA analyzed from a peripheral blood sample was shown. This confirmed the diagnosis of LHON.

Pulse corticosteroid therapy was administered for five days at a dose of 1 g, after which there was a significant improvement in visual acuity. Visual acuity in both eyes was improved to a maximum of 1.0 (200/200) and it was permanently maintained over the next two years of follow-up.

Written consent for publication of the article has been obtained by the patient's family member.

DISCUSSION

In the available literature, this is the first documented LHON case demonstrating complete restitution of visual acuity in both eyes with LHON.

Polymerase chain reaction amplification and sequencing of mitochondrial DNA regions containing mutations m.11778G>A (*MT-ND4*), m.14484T>C (*MT-ND6*), m.3460G>A (*MT-ND1*) have been shown to occur in about 90% of patients with LHON.

In a study by Mashima et al. [6], the effect of idebenone (Raxone; Santhera Pharmaceuticals Holding, Pratteln, Switzerland) was monitored in patients with LHON. Twenty-five (20.5%) of the 122 eyes had a recovery of their visual acuity to ≥ 0.2.

In a study by Newman et al. [7], among 695 patients with LHON, in patients with the m.11778G>A mutation, recovery of meaningful vision likely occurs in less than 20% of patients, irrespective of how recovery is defined, and ultimate visual acuities of better than 20/200 are rare.

The m.11778G>A LHON patients treated with gene therapy rAAV2/2-*ND4* exhibited an improvement of visual acuity over more than four years after vision loss to a degree not demonstrated in natural history studies [8].

Options for the effective treatment of hereditary optic neuropathies have been a long time coming. The successful launch of the antioxidant idebenone for LHON, followed by its introduction into clinical practice, was an important step forward. Nevertheless, other options, especially for a variety of mitochondrial optic neuropathies, such as dominant optic atrophy, are needed, and a number of pharmaceutical agents, acting on different molecular pathways, are currently under development. These include gene therapy, which has reached Phase III development for LHON [9].

Our case advocates that there is no secure treatment for visual outcome in patients with LHON. In our experience, introducing pulse corticosteroid therapy as soon as possible is highly recommended in LHON patients.

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Леберова хередитарна оптичка неуропатија са потпуним опоравком видне оштрине – први приказ болесника

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САЖЕТАК

Увод Леберова хередитарна оптичка неуропатија (ЛХОН) типично погађа млађе људе са већом преваленцом код мушкараца, али може се десити у било ком животном добу и такође код жена. ЛХОН настаје због мутације у митохондријалној ДНК. Класично се презентује са субакутним унилатералним губитком вида, дисхроматопсијом у црвено-зеленом спектру и централним или центроцекалним скотомом. Друго око обично развија сличне симптоме за три до шест месеци од почетка болести.

Приказ болесника Болесник старости 55 година дошао је на преглед код неуролога 20 дана након изненадног губитка вида. Одмах је упућен као хитан случај на Клинику за очне болести због изненадног губитка вида на оба ока. Најбоље коригована видна оштрина на оба ока је износила 4/60. Интраокуларни притисак је био нормалан на оба ока. Едем главе оптичког нерва је био присутан на десном оку, а на левом се нејасно ограничавала глава оптичког нерва. Током хоспитализације је урађено више различитих консултативних прегледа и дијагностичких процедура, заједно са лабораторијском анализом крви и компјутеризованим видним пољем. Урађена су генетска тестирања на ЛХОН, као и антитела за *AQ4*, имуносерологију, вирологију и лумбалну пункцију. Урађени су такође визуелни евоцирани потенцијали – ВЕП и ултразвучни преглед.

Закључак Код нашег болесника је пронађена хетероплазматска мутација *m.11778 G>A* (*MT-ND4*) у митохондријалној ДНК добијеном анализом узорка периферне крви. Прегледом доступне литературе, ово је први документовани случај ЛХОН који показује комплетан опоравак видне оштрине на оба ока са ЛХОН.

Кључне речи: Леберова хередитарна оптичка неуропатија; изненадни губитак вида; митохондријална мутација ДНК

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Stress and arterial hypertension – from pathophysiology to pharmacology

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SUMMARY
Currently, arterial hypertension is the most massive chronic non-infectious disease of mankind. It may remain undiagnosed for years, provoking later complications, such as acute heart failure, cerebrovascular stroke, myocardial infarction, renal failure, hypertensive retinopathy, or sudden death. Primary arterial hypertension is more common, while secondary occurs in about 5–20% of cases. The recent studies have shown that stress may be a core factor in the development of essential hypertension in some patients. For the patients suffering from post-traumatic stress disorder, stress is the dominant etiological factor that leads to the disease. It has been proven that chronic stress can affect blood pressure regulation and endocrine-metabolic functions through the limbic-hypothalamic centers; therefore, it can affect the arterial hypertension development. The strong association between stress and arterial hypertension has also been confirmed in preclinical and animal studies. For the pharmacotherapy approach, the most important are beta-adrenergic blockers, angiotensin-converting enzyme (ACE) inhibitors and AT1-receptor blockers (sartans). As a second line treatment, calcium channel blockers, diuretics, alpha-adrenergic blockers, and central antihypertensive agents may be required. The anxiolytics, such as benzodiazepines, should be considered if chronic anxiety and psychosomatic disorders are present.
Keywords: stress; arterial hypertension; therapy; anxiolytics

INTRODUCTION

Arterial hypertension (AH) represents the most common illness from the cardiovascular diseases (CVD) group, and, according to the latest data from the World Health Organization, 1.13 billion people worldwide are suffering from it, while every fourth male and every fifth female suffered from AH in 2015. In such context, AH as a contributing factor of CVD is the most massive chronic non-infectious disease of mankind nowadays [1]. AH can be primary and secondary; primary is far more common (approximately 80–95%) and the cause is unknown, while secondary occurs in about 5–20% of cases and occurs as a consequence of other illnesses [2].

AH represents the most common risk factor for CVD. A study from March 2021 concluded that even stage 1 hypertension defined by the American College of Cardiology and American Heart Association guidelines was independently associated with subclinical coronary atherosclerosis [3]. In the United States, considerably higher prevalence of AH has been noted in African Americans compared to other races. Thus, in a recent study conducted on a community-based cohort of African Americans, it was concluded that higher perceived stress over time is associated with an increased risk of developing hypertension [4].

Many organs participate in stress reactivity; however, the essential role is played by the hypothalamic-pituitary-adrenal (HPA) axis with corticosteroid secretion, as well as the neurovegetative system and the adrenal medulla with consequent secretion of catecholamines [2, 5].

ARTERIAL HYPERTENSION AND STRESS

Stress has been noted in SCORE system as one of the contributing factors to CVD risk in the European Society of Cardiology / European Society of Hypertension guidelines for 2018 (Table 1). Blood pressure (BP) represents a circulatory parameter, which is controlled by baroreceptors. When BP rises, it affects the baroreceptors, which are most densely distributed in the bulbus of the carotid artery and the aortic arch, and their main characteristic is that they are sensitive to stretching. Stretching caused by an increase in BP leads to transmission of information by baroreceptors along the vagal and glossopharyngeal pathways toward the solitary nucleus in the brainstem, which makes single and multiple neural connections to pre-autonomic source nuclei in the

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Table 1. Risk modifiers that increase cardiovascular risk estimated by the Systemic Coronary Risk Evaluation

Social deprivation, the source of many causes of cardiovascular diseases
Obesity (according to the body mass index) and central obesity (measured by waist circumference)
Physical inactivity
Psychosocial stress, including vital exhaustion
Family anamnesis of early cardiovascular disease (before the age of 55 in men and before the age of 60 in women)
Autoimmune and other inflammatory disorders
Major psychiatric disorders
HIV infection treatment
Atrial fibrillation
Left ventricular hypertrophy
Chronic kidney disease
Obstructive sleep apnea syndrome

brainstem and also to the forebrain. These structures have crucial role in regulating BP. Psychological stress has been shown to reliably reduce baroreflex sensitivity, specifically cardiovagal sensitivity [6].

Studies conducted on animal and human models have found that the network of cortical areas, limbic system and brainstem plays an important role in generating and regulating stress-provoked cardiovascular reactivity. It should be noted that from the pathophysiological aspect, stress-induced cardiovascular reactions are a consequence of changes in the sympathetic and parasympathetic nervous systems as well as in the HPA axis, which act on the heart and vasculature. Recent research has shown that higher levels of amygdala activity in rest predict the development of CVD over a period of 3.7 years. Increased amygdala activity is associated with changes in immune activity, and also with arterial inflammation and perceived stress, which provides evidence of potential pathways that support the development of CVD [7]. An animal model study published this year also confirms the link between stress and AH, with the very interesting conclusion that V1a and V1b receptors for vasopressin within the paraventricular nucleus contribute to hypertension in male rats exposed to chronic mild unpredictable stress [8].

The function of the HPA axis can be evaluated by measuring cortisol in the blood, saliva or urine. The recent data suggests the measurement of cortisol levels in the hair as a new biomarker of long-term HPA axis activity [9]. Some of the mechanisms that explain the development of cortisol-induced hypertension include its mineralocorticoid action in the form of sodium retention, then the expansion of plasma volume and inhibition of vasodilatory hormones [9]. There are several studies that have studied the ways in which stress can affect the epigenetic regulation of the HPA axis, so one of them states that DNA methylation of genes involved in the regulation of glucocorticoids is associated with AH and subclinical atherosclerosis [10]. Short sleep and discontinuous rest, which are often found in women with depression, disrupt balance of the sympathetic and parasympathetic nervous system and are associated with hypersecretion of cortisol, thus increasing heart rate and BP [11]. Furthermore, it is concluded that the intestinal microbiome disturbance, which can be a consequence of stress, is associated with AH, CVD, and metabolic diseases [12].

A study by Rao et al. [13] demonstrated that adrenergic polymorphism affects the human response to stress, and thus BP levels and catecholamine secretion are influenced by genetic variation of the adrenergic pathway encoding catecholamine synthesis, specifically a step that limits the rate of synthesis, which is the enzyme tyrosine hydroxylase, or a genetic polymorphism for the said enzyme. Arosio et al. [14] proved the existence of influence of mental stress on AH, but also the protective effect of AT1-receptor blockers on noradrenergic and adrenergic stress in hypertensive individuals.

The studies concerning stress and AH have been also conducted in animal models. Earlier publications have already established a cause-and-effect relationship between posttraumatic stress disorder (PTSD), where stress is the dominant etiological factor leading to the disease and AH. This was also confirmed in a recent study by Xue et al. [15].

PHARMACOLOGICAL ASPECTS

The specificity of stress-induced AH is that in addition to antihypertensive therapy, drugs that affect the patient's mental status, such as anxiolytics, may be recommended. There are non-pharmacological forms of treatment in the form of psychological support and psychotherapeutic techniques (relaxation techniques, stress management techniques, suggestion techniques, positive thinking and visualization techniques) [16]. For the pharmacological measures, there are several groups of antihypertensive drugs available. The most important ones, when treating stress-induced AH, are beta-adrenergic blockers, angiotensin-converting enzyme (ACE) inhibitors and AT1-receptor blockers (sartans). In case of resistant AH, calcium channel blockers, diuretics, alpha-adrenergic blockers, and central antihypertensive drugs may be required. Moreover, some studies recommend that patients with impaired autonomic activity and stress-induced AH be genetically profiled in relation to adrenergic pathways, and if a genetic risk is identified, it is considered that these patients would benefit from sympatholytic therapy [13]. The type of sympatholytic therapy most frequently mentioned in the literature recently is renal denervation. It is a minimally invasive therapeutic method based on catheter radiofrequency (although ultrasound or alcohol injection may be used) ablation of afferent and efferent renal sympathetic fibers, which is usually reserved for severe AH resistant to pharmacological treatment and AH with concomitant chronic renal failure, although it has recently been suggested to expand the indications to uncomplicated AH [17, 18, 19]. The first study that proves the reduced efferent renal sympathetic innervation after chemical renal denervation in humans, as well as the positive effects of this procedure on AH, was published in March 2021 [20].

Beta-adrenergic receptor blockers are not the first in line for antihypertensive therapy, due to the lower antihypertensive effect compared to some other antihypertensives,

because of the negative chronotropic effect on the myocardium, which is not desirable in all hypertensive patients, as well as due to the effect on beta2-adrenergic receptors in non-selective blockers, thereby reducing insulin secretion. However, for stress-induced AH, in addition to the antihypertensive effect, beta-blockers also have an effect on reducing tachycardia, which is almost always present in these patients. These drugs antagonize the action of catecholamines and partially lead to the patient's relaxation and stress reduction.

The effect of ACE inhibitors is reflected in arterial and venous dilatation, reduction of peripheral vascular resistance, increase in minute volume, effort tolerance, the excretion of sodium and water by the kidneys, preventing the proliferation of the smooth muscle cells, reduction of the left ventricular hypertrophy. They have an important role in AH therapy, but also in cases of stress-induced AH [15]. The indications spectrum of AT1-receptor blockers (sartans) is identical to ACE inhibitors. They are introduced into therapy in case of intolerance to ACE inhibitors; dry cough as a consequence of bradykinin action caused by the action of ACE inhibitors, or reduced ACE inhibitors efficiency in situations of elevated plasma renin. The justification of their use in stress-induced hypertension can be found in the conclusion of the study by Arosio et al. [14], where it has been shown that AT1-receptor blockers act protectively in noradrenergic and adrenergic stress in hypertensive patients.

Calcium channel blockers lead to smooth muscle arteries cells relaxation, vasodilation, reduction of peripheral vascular resistance, and lowering BP. However, these drugs are not the first in line when treating stress-induced hypertension. It is similar in regard to diuretics and alpha-blockers, which have no significant application in this case, except when dealing with resistant stress-induced AH.

The use of anxiolytics should be considered only in the case of chronic anxiety and psychosomatic disorders. There are studies that indicate a favorable impact of GABAergic systems' modulation in the treatment of anxiety with related CVD [21]. Benzodiazepines, as positive GABAergic modulators, are often prescribed with the internal medicine therapy and added to the treatment of chronic hypertension. Both quantitative and qualitative consumption data have confirmed this in practice [22]. The advantages of benzodiazepines are a relatively safe pharmacological profile and a low risk of serious side effects; however, they can produce tolerance and dependence after long-term treatment. The hypertension pharmacotherapy guidelines do not recommend routine use of benzodiazepines except when associated with psychiatric comorbidities. Especially in the older population, simultaneous use of benzodiazepines has been shown to increase the risk of limb fractures and injuries, as well as reduced cognitive abilities [23]. Some recent experimental studies have indicated that certain benzodiazepines, like midazolam and diazepam, can be considered. It has been shown that midazolam induces arterial blood vessels vasodilation, most likely by voltage-gated calcium channel modulation, while diazepam exerts its effect by alpha-1 receptor modulation [24, 25]. However, their efficacy has neither been confirmed by meta-analyses nor by long-term follow-up studies.

CONCLUSION

Nowadays, AH represents the most widespread, non-infectious disease of mankind. Chronic stress represents an increasingly common etiological cofactor, while in some situations it is the main cause of AH. Thus, it is necessary to consider and evaluate the presence of stress in the modern treatment. The decision which pharmacological agents shall be introduced into therapy depends on comorbidity, other CVD risk factors, and the patient's age. There is a need to primarily introduce beta-adrenergic blockers, ACE inhibitors, or sartans for the stress-induced AH, while the use of anxiolytics should only be considered if chronic anxiety and psychosomatic disorders are present.

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Стрес и артеријска хипертензија – од патофизиологије до фармакологије

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САЖЕТАК

Артеријска хипертензија је данас најмасовнија хронична незаразна болест човечанства. Може остати недијагностикована годинама, што изазива касније компликације, попут акутне срчане инсуфицијенције, можданог удара, инфаркта миокарда, бубрежне инсуфицијенције, хипертензивне ретинопатије или изненадне смрти. Примарна артеријска хипертензија је чешћа, док се секундарна јавља у око 5–20% случајева. Недавна истраживања су показала да стрес код неких пацијената може бити кључни фактор у развоју есенцијалне хипертензије. Код пацијената који пате од посттрауматског стресног поремећаја, стрес је доминантни етиолошки фактор који доводи до болести. Доказано је да хронични стрес може да утиче на регулацију крвног притиска, ендокрине и метаболичке функције путем лимбичко-хипоталамичких центара и самим тим да утиче на развој артеријске хипертензије. Снажна повезаност стреса и артеријске хипертензије потврђена је у претклиничким студијама и испитивањима на животињама. За фармакотерапијски приступ најважнији су бета-адренергички блокатори, инхибитори ензима који конвертује ангиотензин и блокатори рецептора *AT*1 (сартани). Као друга линија терапије могу се користити блокатори калцијумових канала, диуретици, алфа-адренергички блокатори и централни антихипертензиви. Увођење анксиолитика, попут бензодиазепина, треба размотрити у случају хроничне анксиозности и психосоматског поремећаја.

Кључне речи: стрес; артеријска хипертензија; терапија; анксиолитици

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Proton beam therapy

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SUMMARY
Proton beam therapy (PBT) is an advanced type of radiotherapy that shows a dosimetric advantage over photon beam therapy and provides superior dose distribution. PBT may improve patient survival by improving the local disease control while reducing toxicity to normal organs, which may result in fewer treatment-related complications. During the last decade, technological progress has opened up new possibilities in the planning and conducting of PBT, so indications have gradually expanded to different cancers. However, many biological aspects of PBT are still unclear, and its role in clinical settings is controversial. Proton therapy is considered to be safe and effective for different types of pediatric cancers, and suitable in treatment of ocular melanomas, chordomas, and chondrosarcomas. Future research and more prospective clinical studies with long-term follow-up are required in order to clearly determine the benefits and proper indications for PBT.
Keywords: proton therapy; radiotherapy; cancer

INTRODUCTION

Proton beam therapy (PBT) is a modern radiotherapy (RT) technique that uses protons. In 1946, Wilson first proposed PBT for medical use considering the advantages of proton RT compared with conventional photon RT. This suggestion was based on the known physical property of protons, which is that they slow down during penetration of tissue [1, 2].

The first PBT patient series was published in 1958 by researchers at the Lawrence-Berkeley National Laboratory, where patients with radio-resistant tumors such as chordoma and melanoma were initially treated. Technological progress opened up new possibilities in PBT planning and conducting, so indications were gradually expanded to other cancers. The expenses of PBT are much higher compared to conventional photon RT due to the high cost of proton beam technology and maintenance. First proton center was established in 1990 in California, and today there are about 70 proton therapy centers worldwide with more than 190,000 patients treated with PBT [3, 4].

Increasingly more evidence has been showed for the advantages of PBT in clinical use, but it is not suitable for every tumor type and site. Also, some biological aspects of PBT are still unclear. It is necessary to understand the advantages and limitations of protons [5].

Physical and biological aspects of PBT

Protons are heavy charged particles which continuously slow down during penetration of matter as they slow down in a function of depth. Energy loss continues until the entire energy of the proton is depleted, after which they come to an abrupt stop, which results in a steep and localized peak of dose. This process of dose deposition produces a characteristic depth-dose curve – the Bragg curve. The point of the highest energy loss of proton is called the Bragg peak (Figure 1). The depth of the peak depends on the initial proton energy, and the deposited dose beyond the range is minimal. PBT dose distribution is superior to the dose distribution of conventional photon RT, but it is still debatable whether the dosimetric advantages of PBT translates to clinically relevant decreases in toxicity. Different randomized clinical trials which compare protons and photons are currently ongoing [2, 6].

The proton dose is defined as gray (Gy), which is calculated by multiplying the physical dose by the relative biological effectiveness (RBE). For photon and electron external beam RT, the RBE is considered to be 1. Proton RT is planned assuming that the proton RBE relative to photons is 1.1. However, experimental

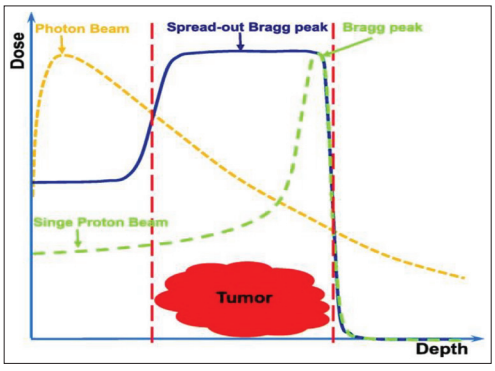

Figure 1. The diagram of dose distributions for photon, single proton beam, and spread-out proton beam [5]

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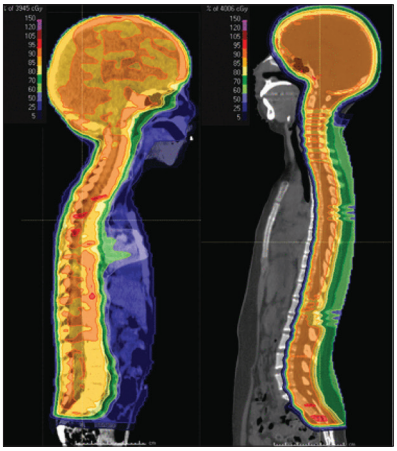

Figure 2. Dose distributions for photon (left) and proton (right) craniospinal radiotherapy plan [12]

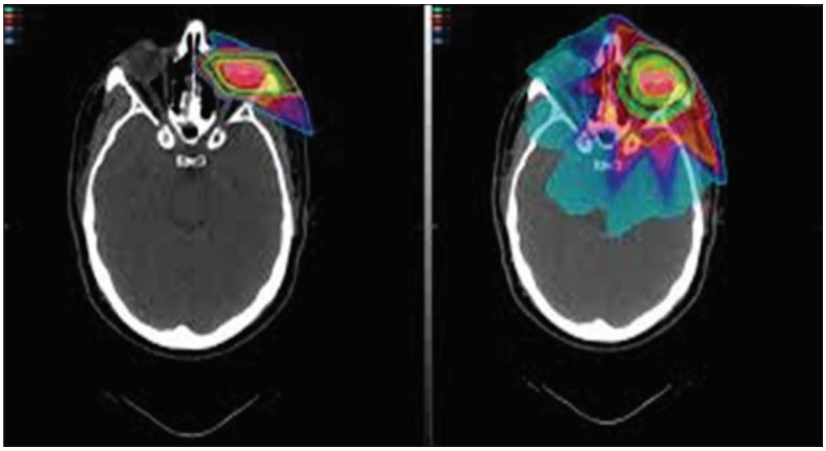

Figure 3. Isodose distributions for proton (left) and photon (right) treatment plans for ocular melanoma [18]

evidence showed that proton RBE is not constant and that it changes along the treatment field. According to *in vitro* studies, the highest RBE is found at the distal edge and in the distal fall-off region within the Bragg curve. Still, there remain several uncertainties in understanding variations in biological response after proton irradiation compared to photon irradiation. Current experiments on the response of normal and tumor tissue to proton therapy should be continued [7].

Proton therapy for different cancers

The heavier subatomic particles deliver their energy more precisely to the tumor area compared to photons. The justification for the clinical use of proton therapy is the possibility for dose escalation to the tumor, which leads to better local disease control probability. This is possible due to better sparing of surrounding healthy tissue compared to other RT techniques. Due to the reduced treatment volume and a lower integral dose, patient tolerance is increased with lower morbidity rate. PBT may improve the survival rate with significant reduction of treatment-related complications, which results in preserving the quality of life of treated patients.

As other highly conformal photon therapy techniques, PBT is indicated for tumors located close to serial organs, where a small radiation overdose can lead to severe complications. Irregular shaped lesions close to critical structures are suitable for proton RT treatment [3, 8].

Pediatric cancers

Radiation therapy plays an important role as part of multimodal treatment for many pediatric malignancies, especially for brain tumors, sarcomas, lymphomas and neuroblastoma. Treating children with RT is a great challenge because they have higher radiation sensitivity and lower radiation tolerance than adults, and late toxicity of RT is an issue for long-term survivors. Reduction in the quality of life due to growth and development retardation, as well as secondary malignancies, remains a significant problem for treated children. It is necessary to provide effective radiation therapy with the least possible morbidity. The physical characteristics of protons are promising in terms of achieving significant clinical benefits [9, 10].

Dosimetric comparation studies between photons and protons in treatment of medulloblastoma, ependymoma, Ewing sarcoma, rhabdomyosarcoma showed the superiority of PTB over photons in reducing dose to surrounding healthy organs and tissues (Figure 2). Clinical results are limited, but the first evidence confirmed similar survival rates with fewer treatment-related side effects for PBT, which could have positive impact on the quality of life of treated children [11].

Gross et al. [13] reported favorable neurocognitive outcomes in pediatric patients with brain tumors with the use of PBT compared with photon RT, according to findings from a study that included 125 patients.

Kahalley et al. [14] published the first longitudinal study comparing intellectual outcomes between pediatric patients treated for medulloblastoma with PBT and photon RT, and showed that PBT was associated with superior intellectual outcomes.

On the other hand, Kralik et al. [15] pointed out that pediatric patients with brain tumors treated with PBT have a high incidence of radiation necrosis, frequently distant from the tumor area. Multiple chemotherapy agents were significant risk factors associated with radiation necrosis.

Bhattacharya et al. [16] did a retrospective imaging review of 46 patients with brain tumors treated with PBT. Large vessel progressive cerebral arteriopathy was described in 25% of patients, which is more than in previously reported studies. This study also pointed out the appearance of white matter changes remote from the region of irradiation in two patients.

There is a need for continued close follow-up of children treated with PBT, which will enable us to better understand long-term effects, safety, and benefits of this therapy.

Ocular tumors

Ocular melanomas represent a perfect model for a malignant tumor requiring high-dose RT with complex dose

distribution within the target volume, and PBT is recognized to be one of the main RT treatment options for these and other ocular tumors [17] (Figure 3).

PBT for ocular melanoma results in excellent local control of disease with preserved quality of life of treated patients. Van Beek et al. [19] published a retrospective study of 306 patients with uveal melanoma. Half of patients were treated with PBT and the other half with fractionated stereotactic photon beam radiotherapy (fSRT). The five-year local tumor control rates were 96.1% for both groups. However, vitreous hemorrhage was significantly less common after PBR than after fSRT.

PBT is also a new option for conservative treatment of conjunctival squamous cell carcinoma. Milazzotto et al. [20] reported a retrospective analysis of 15 patients with conjunctival squamous cell carcinoma treated with PBT who had gross residual disease after surgery or were not candidates for surgery. Overall survival and disease-free survival rates were 86.6% each, after a median follow-up of 48 months Treatment was well tolerated, without significant acute or late toxicity.

Chordoma of the skull base and spine

Chordoma of the skull base is challenging to treat due to tumor location, proximity to critical neural and vascular structures, and tumor radioresistance. Gross total resection of these tumors is often not possible, so adjuvant radiation therapy is an important treatment modality which can improve local disease control and overall survival. High-dose photon-based RT can be used, but usually cannot achieve therapeutic dosage because of the proximity to dose-limiting structures: the optic nerve, chiasm, the brain stem, the spinal cord, and the brain [21].

Application of proton therapy with simultaneous integrated boost for these malignancies made possible the delivery of radical doses to target volumes while minimizing toxicity for organs at risk. This treatment approach affords excellent local disease control while sparing normal surrounding structures [22].

Treatment of spinal and sacral chordoma represents great challenge because of the proximity of the spinal cord and nerve roots. Radiation tolerance of the spinal cord is considered at 48–54 Gy, much below necessary doses adequate for local control for these tumors. Chordoma require high radiation doses of 60–70 Gy. PBT offers a dose escalation for treatment of tumors in this location, but the current clinical evidence is still limited and further research is needed [23].

Reirradiation

Tumor recurrence is in most cases unresectable because of many different factors. The possibility of reirradiation is limited by the previously applied RT treatment, dose constrains for surrounding critical organs, and the time period passed since the previous radiation treatment. The high conformality and rapid fall-off of radiation dose at the distal end of the target offer significant possibility for reirradiation with protons. By sparing adjacent normal tissues, proton therapy can more safely apply definitive instead of palliative doses of reirradiation [3, 24].

Saeed et al. [25] published a series of 45 patients with recurrent glioblastoma multiforme treated with proton reirradiation 2012–2018. The median interval between initial diagnosis and disease recurrence was 20 months. In this series, 40 patients completed full reirradiation course with a median dose of 46.2 Gy. The median progression-free survival was 13.9 months with median overall survival of 14.2 months. One grade 3 acute toxicity was observed, three patients developed grade 3 late toxicity, and no grade 4 or 5 toxicities were reported.

Although a small number of published studies on reirradiation with PBT have shown promising results, adequate patient selection is required for the careful use of proton reirradiation.

Other tumors

PBT has been used for treating different malignancies, including central nervous system, head and neck tumors, prostate, breast, liver, esophageal, and lung cancer. However, the role of PBT in clinical settings is still controversial, and there are certain technical challenges in planning and delivery for different treatment sites [5].

CONCLUSION

PBT is an advanced type of RT that achieves a dose distribution generally superior to photon beam therapy. This may allow dose escalation to the tumor target volume, better sparing of surrounding tissues, thus potentially improving local disease control and survival while at the same time reducing toxicity and improving the quality of life of treated patients. Still, a question remains as to whether dosimetric advantages of PBT leads to clinically relevant decreases in toxicity. Clinical evidence supporting wide use of protons is mixed despite its high potential. Promising results have been reported for many types of cancers; however, they are based on small studies. There are still uncertainties about the radiobiology of protons that can have an impact on the molecular and cellular effects of PBT. Further research and prospective clinical studies with extensive follow-up of treated patients are needed in order to determine effectiveness and safety of PBT.

Conflict of interest: None declared.

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Протонска терапија

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САЖЕТАК

Протонска терапија је напредна радиотерапијска техника која показује супериорнију дозну дистрибуцију и дозиметријску предност у односу на радиотерапију фотонима. Протонска терапија може побољшати преживљавање болесника омогућавањем боље локалне контроле болести уз смањено зрачење околних здравих органа, што резултира нижом стопом терапијских компликација. Током претходне деценије технолошки напредак довео је до нових могућности за планирање и спровођење протонске терапије, те је порасла њена примена у третману различитих тумора. Међутим, биолошки аспекти протонске терапије још увек нису разјашњени, а клиничка примена је и даље контроверзна. Сматра се да је протонска терапија безбедна и ефикасна у третману различитих педијатријских тумора и да је адекватна у случајевима окуларног меланома, хордома и хондросаркома. Неопходна су даља истраживања и проспективне клиничке студије са дугорочним праћењем болесника како би се јасно утврдиле предности и одговарајуће индикације за примену протонске терапије.

Кључне речи: протонска терапија; радиотерапија; канцер

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INVITED REVIEW ARTICLE AND CALL FOR ACTION
/ ПРЕГЛЕДНИ РАД ПО ПОЗИВУ И АКЦИОНИ ПЛАН

Women's health in Serbia – past, present, and future

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SUMMARY
Cardiovascular and reproductive health of women have been going hand in hand since the dawn of time, yet links have been poorly studied. Still, once basis of their connections started to be established, it depended on local regional abilities and level of progressive thinking to afford women comprehensive care beyond the "bikini medicine".
Further research identified different associations rendering more conditions sex-specific and launching therefore a slow, yet initial turn around in clinical trials' concept as the majority of global cardiovascular guidelines rely on the results of research conducted on a very modest percentage of women and even less on the women of color.
Currently, the concept of women's heart centers varies depending on the local demographics' guided needs, available logistics driven by budgeting and societal support of a broad-minded thinking environment, free of bias for everyone: from young adults questioning their gender identity, via women of reproductive age both struggling to conceive or keep working part time when healthy and line of work permits it during pregnancy, up to aging and the elderly.
Using "Investigate-Educate-Advocate-Legislate" as the four pillars of advancing cardiovascular care of women, we aimed to summarize standing of women's health in Serbia, present ongoing projects and propose actionable solutions for the future.
Keywords: COVID-19; sex differences; pregnancy; women in cardiology; discrimination; diversity; inclusion

INTRODUCTION

Women's health has traditionally been following the "bikini medicine" concept making women live in a delusion that breast cancer is their biggest enemy, when in fact it is heart disease that claims more lives than all cancers combined. Not only do we see these disparities in cardiovascular care in the United States, the beacon of modern democracy and global leader in technologically savvy healthcare, but worldwide also, where women's access to care and policies vary extensively rendering all implementation of innovative ways more challenging [1, 2]. The ongoing black maternal crisis in the United States, sadly, confirmed that, even in most affluent settings, black mothers suffer more than other women during childbirth and the "fourth and fifth trimester", so care offered to women has to become void of all bias [3, 4]. The bias-free care is taught in medical schools in different forms, however, sex-specific pathologies and racial disparities in a world whose landscape changes due to migrations of all kinds, demand our attention to the most vulnerable to meet the demand of the patients we tend to treat to the best of our knowledges [5]. The women's heart centers (WHC) and programs in Northern America have been established over the past three decades, however, their concept and structure remain a work in progress globally [6, 7].

DEMOGRAPHICS

Per last available 2019 edition of the "Health Statistical Yearbook of the Republic of Serbia", published by the Institute of Public Health of Serbia, "Dr Milan Jovanović Batut", showed a 3.4% population decline from the 2011 census with a total of 3,561,503 women representing 51.3% of the population [8]. Same source reports that the women of childbearing age (15–49 years) make 21.7% of the population, while the aging and the elderly (over 65 years of age) make 20.7%. Steady decline in birth rates was noted over the past decade, while an additional 2.3% drop was registered when comparing January to July of 2020 and 2021, the two COVID-19 pandemic years [9].

SEX-SPECIFIC MORBIDITY AND MORTALITY IN SERBIA

At the primary healthcare level 5731 women over 15 years of age are assigned to one gynecologist, and in 2019, 43% of the visits represented first time ever visits and the prevailing reasons of visits were disease of the genitourinary tract (36%), pregnancy (6%) and tumors (4%) [8].
Currently, approximately half of the women of reproductive age struggle with some fertility issue from dysmenorrhea, polycystic ovary syndrome via infertility [8, 9] necessitating repeated assisted reproductive technology, known to increase cardiovascular risk long term [10, 11], besides a myriad of different acute complications of the procedure.

ACTIONABLE SOLUTIONS

Using the ***"Investigate-Educate-Advocate-Legislate"*** as the four pillars of advancing cardiovascular care of women [12], we aimed to summarize both the standing of women's reproductive and cardiovascular health Serbia and actionable solutions

Investigate

Reviewing currently available sex-specific research dedicated to women's health in Serbia, a pattern itself is absent and reported results come mainly from a moderate number of centers and research groups: whether from basic research standpoint [13–16], or clinical ones dedicated to women's cardiometabolic health [17–21], cardio-obstetrics [14, 22, 23, 24], peri-/menopause [25, 26, 27], different cardiovascular outcomes [28, 29], cancer [30, 31, 32] or mental health [33, 34] including the vulnerable and underserved populations [35].

Under-representation of women in recruitment practices and delayed invasive strategy in Serbia – *per local, in press and unpublished results of ongoing research projects* – are detrimental as anywhere else in the world, however day-to-day re-evaluation of our changes of clinical practices remain of critical importance. The COVID-19 pandemic opened an additional Pandora's box of suboptimal care for women [36] and women frontline healthcare workers in

Figure 1. The first "Dr Nanette Kass Wenger" International Conference on CVD in Women (Dec 2018): Program, snapshot of the article dedicated to the event in the Serbian Medical Chamber's journal "Glasnik" and pictures from the event

particular [36, 37, 38], here proposed Serbian model of a WHC offers also a possible solution to the problem.

Also, more comprehensive registry-oriented and epidemiological research of different population strata should ameliorate long term research and, consequently, improve outcomes for women in Serbia at all levels of care currently offered both in nationally budgeted institutions (primary, secondary and tertiary), as well as privately-owned ones (primary mainly, secondary in a small number of selected procedures).

Educate

The road to providing sex-specific education for medical students is seemingly a long and winding one, even in countries where budgeting is not an issue and multiple sources of funding are available. However, before the new generations of physicians reach the workforce, continuing medical education for sub-specialties beyond endocrinology, gynecology and obstetrics, and urology, is sorely needed together with implicit bias trainings along with recertification. Although dedicated WHC culturally, worldwide, are staffed with women, teaching male doctors that "women are not small men", but that their presentation of the same diseases can be different, as well as that with current migration rates, women of different ethnic [39] carry different burden of disease is of a growing importance.

Furthermore, educating women as patients of their symptoms and risks for different outcomes is equally important.

Advocate

Aiming to promote awareness and advance cardiovascular care of women in Serbia, the "Dr Nanette Kass Wenger" International Conference on Cardiovascular Disease in Women, was born as an idea to help bridge the gaps in available regional knowledges in the least judgmental way and help existing local teams build bonds.

The launch of the conference series started in 2018 supported by the current Presidents of the American Heart Association, Dr Robert A. Harrington and the American College of Cardiology's Dr Michael Valentine and both associations' CEOs, Ms Nancy Brown and Ms Cathy Gates. International speakers: Drs Sandra Lewis, Annabelle Santos Volgman and Martha Gulati were welcomed by the ACC Chapter Governor of Serbia and Republika Srpska Professor Milan Nedeljković, Belgrade University's School of Medicine Dean Academician Nebojša Lalić, the Serbian Medical Chamber's Director Dr Milan Dinić and Academician Vladimir Kanjuh, president of the Serbian Academy's Science and Arts' Board for Cardiovascular Pathology who was one of the co-organizers (Figure 1). The newly confirmed Government, led by Ms Ana Brnabić, voiced its support to formation of a WHC under the roof of the leading country's healthcare institution, the University Clinical Center of Serbia, in an appeal sent out to needed medical and scientific entities by the Minister for Population Policy and Demographics Dr Slavica Đukić-Dejanović – *a professor of Psychiatry and fervent advocate of equitable healthcare for the underserved populations, besides formal engagement with the national UN's Generation Equality initiative* – who stressed the importance of timely management of healthcare issues of women and girls, and the first to confirm support was Academician Vladimir Kostić, President of the Serbian Academy of Sciences and Arts.

The second conference took place in December 2019 where besides sustained local and international support, the launch of the THEMIS foundation announced partnership with American Heart Association's GoRedForWomen for Serbia and additional support from the Royal Medical Board was offered, while Professor Wenger was hosted by the Crown Princes Alexander and Katherine. During her working visit, Dr Wenger participated in multiple meetings with local opinion leaders in the field, addressed a reunion of national nursing leadership and also met with the representative of the National's Gender Equity office that reaffirmed Government support in providing equitable care (Figure 2). The existing support of the Minister of Health, Associate Professor Dr Zlatibor Lončar, was confirmed once more and Dr Wenger toured the entire campus of the University Clinical Center of Serbia under heavy reconstruction at the time, while the idea of offering virtual telemedicine visits was emerging already.

As the world came to a standstill that delayed all care to all other patients with the emerging SARS-CoV2 pandemic in Europe in late 2019 and early 2020 in Serbia, the physical opening of the WHC planned for later that year suffered

Figure 2. The second "Dr Nanette Kass Wenger" International Conference on CVD in Women (Dec 2019):
(a) Professor Gordana Teofilovski-Parapid (Member of the Board for cardiovascular pathology of the Serbian Academy of Sciences and Arts), together with Academician Professor Dragan Micić and Professor Snežana Polovina hosting Professor Wenger at the Serbian Academy of Sciences and Arts
(b) Program of the event
(c) Professor Wenger with the Crown Princes' Alexander and Katherine and Ms Danijela Šegan, representative of the Gender equity office of the Serbian government (sitting) with Professor Gordana Teofilovski-Parapid, Dr Biljana Parapid and Professor Lukas Rasulić (standing) during the THEMIS Foundation launch and presentation of the GRFW program for Serbia

Figure 3. The third "Dr Nanette Kass Wenger" International Conference on CVD in Women (Dec 2020): Program and invite, while link of the event is available in the references

a delay, as well. However, the pilot telemedicine projects with the one of the oldest Belgrade's Primary Healthcare Physician's Office Centers, led by Dr Branka Lazić in collaboration with Dr Parapid, went as planned with minimal modifications for the benefit of the patients. So, the third conference amidst pandemic was held in December 2020 and hosted virtually [40] courtesy of the International Society of Telemedicine and eHealth, Working Group for Telecardiology President Dr Alexandru Mischie and Co-Chaired by ESC's Board Member Professor Dan Gaita, Professor Aleksandar Nešković as Cardiology Fellowship Program Director of the University of Belgrade, Faculty of Medicine and Dr Parapid as founder of the Conference series and WHC. Faculty of previous two conferences were joined by Dean Emeritus of the Georgetown University School of Medicine Professor S.Ray Mitchell that couldn't be hosted live for the Belgrade University's 100th birthday as planned and also by growing number of global allies of the "Dr Nanette Kass Wenger" WHC in Belgrade who together confirming the toll of the SARS-CoV2 pandemic and its influence on women's health on both sides of the frontline that was noted earlier [36] and that later reconfirmed in Serbia as well [37] only increased the need of such Center. Also, the 2020 conference gained another important global ally in the *Women As One* think tank whose team helped promote the event and committed to further partnership

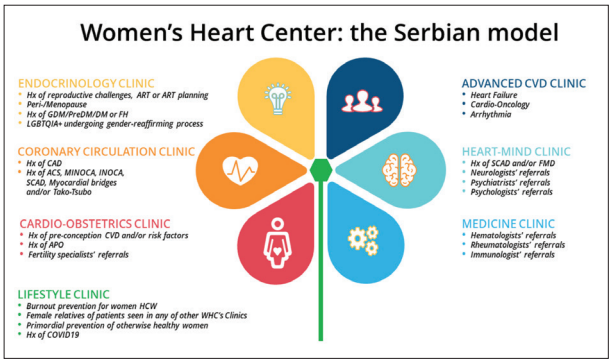

Figure 4. The "Dr Nanette Kass Wenger" Women's Heart Program and Women's Heart Center (Belgrade, Serbia) outline; Hx – history; GDM – gestational diabetes mellitus; DM – diabetes mellitus; FH – family hypercholesterolemia; LGBTQIA+ – lesbian, gay, bisexual, transgender, queer/questioning (one's sexual or gender identity), intersex, asexual/aromantic/agender and allies; CAD – coronary artery disease; ACS – acute coronary syndrome; MINOCA – myocardial infarction with non-obstructive coronary arteries; INOCA – ischemia and no obstructive coronary artery disease; SCAD – spontaneous coronary artery dissection; CVD – cardiovascular disease; APO – adverse pregnancy outcomes; HCW – healthcare workers; WHC – Women's Heart Center; FMD – fibromuscular dysplasia

in all equity-dedicated endeavors of the conference and "Dr Nanette Kass Wenger" WHC team (Figure 3).

The fourth conference planned for December 2021 should be a hybrid one: local limited seating event aiming to support the fully vaccinated COVID-19 pandemic healthcare workforce that is attempting to re-prioritize care of all patients beyond the SARS-CoV2 and virtual involvement of all interested, but mainly foreign faculty with travel restrictions.

As shown in Figure 4, we propose a model of a women's heart center most suiting the needs of a country on the road of financial recovery, encompassing multiple clinics:

Endocrinology clinic

Endocrinology and Reproductive Endocrinology play an important role in cardiovascular risk factors' management of women of different ages and life stages [41]: from safe peri-conception counselling of apparently healthy women, via assisted reproductive techniques (ART) management [42], menopause/hormone replacement therapy [43] and in sex-reaffirming process due to described risks for accelerated development of heart disease [44]. Existing teams of the Division of endocrinology, diabetes and metabolic disorders of the University Clinical Center of Serbia would continue its collaboration with the WHC's team.

Medicine Clinic

Hematologists', Rheumatologists', Immunologists' and Geriatrics' referrals locally constitute the second largest group of women patients necessitating cardiologists' follow up at least during a short period of the treatment received in respective sub-specialty clinics or as hospitalized patients whose management is beyond the usual interdepartmental cardiology consult offered by the Division of Cardiology. Formalization of existing collaboration between research groups of other teaching hospitals of the University of Belgrade, Faculty of Medicine was delayed due to COVID-19 pandemic, but is on the way.

Coronary Circulation Clinic

It is well established [45] that non-obstructive coronary disease is more common in women, including myocardial infarction with no obstructed coronary arteries (MINOCA) [46], ischaemia without obstructed coronaries (INOCA) [47], spontaneous coronary artery dissection (SCAD) [48], fibromuscular dysplasia (FMD) [49, 50], myocardial bridges [51, 52] and Takotsubo (stress-induced) cardiomyopathy [53]. Eventually, when hospitalized [54, 55], women receive far less invasive treatment strategies [56], guidelines directed medical therapy and physical rehabilitation post-discharge with almost immediate return to full activities of daily life in sharp contrast to male patients. Well known gaps in diagnostics [57, 58], care [59] and treatment short- and long-term, need to be bridged [60] and become the standard of care to avoid women being denied timely management for chest pain syndromes.

Heart-Mind Clinic

Diagnoses such as SCAD and FMD are under-diagnosed in Serbia, and when migraine and mental health issues as risk factors for heart disease are added, all remain under-studied and consequently, patients under-treated. The Clinic would offer an umbrella for nation-wide programs with pre-existing partners, as the relationship of these clinical entities remains subject of ongoing research worldwide [61–66].

Cardio-Obstetrics Clinic

Cardio-Obstetrics as a new field, aims to join Cardiologists and Gynecology & Obstetrics specialists in a collaborative care for women, particularly important where Maternal-Fetal Medicine specialists as such do not exist and where Neonatologists and Pediatricians help the best they can. The clinic is dedicated to women with pre-existing heart disease pre-conception and women who developed an adverse pregnancy outcome (APO) (post-partum cardiomyopathy, preeclampsia, gestational diabetes, premature delivery or infant of low birth weight) during a previous or ongoing pregnancy, but can accept other referrals (Figure 4), in particular from pediatricians aware of the risks children conceived via ART carry, once they reach adolescence [10, 11, 67] and harnessing power of the digital world should become an everyday tool for both children and young adults [68].

Advanced Cardiovascular Disease Clinic

The clinic should see patients of all age groups who present with refractory heart failure of various origins (including non-recovered peri-partum cardiomyopathy, ischemic cardiomyopathy, cancer patients *en lieu* of a separate Cardio-Oncology clinic (if regional needs demand it, an independent one should be opened, taking in consideration overall risks for cancer patient post-procedurally, in general [69]), arrhythmia patients and aging and elderly patients whose pharmacotherapy needs more caution [70–73].

Lifestyle Clinic

Lifestyle intervention for apparently healthy women is a growing global demand irrelevant of patients' age group [70, 74–77], while shift work – even before COVID-19 pandemic – has been confirmed as a risk factor for both cardiovascular disease and infertility [78], however, besides existing [79], specific regional guidance is lacking, while the stratifying cardiovascular risk landscape keeps evolving [80], especially in light of cardiovascular damage post-COVID [81]. The female workforce has additionally faced burnout during the ongoing SARS-CoV2 pandemic [36, 38, 79, 82, 83, 84] emphasizing a rising global need for the institution of a Diversity, Inclusion & Wellbeing Officers or Vice-Deans existing in North America, chiefly. With local governmental efforts and participation in #GenerationEquality initiative of the United Nations – *both by former Minister Dr. Slavica Đukić-Dejanović and*

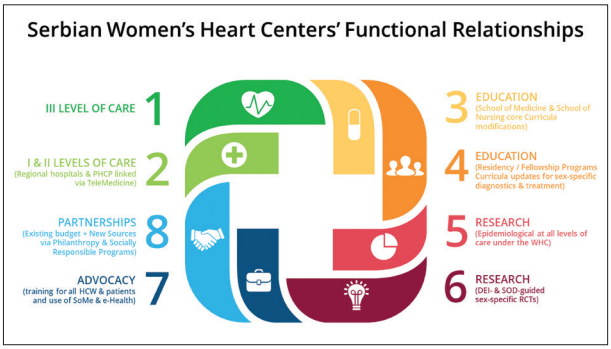

Figure 5. The "Dr Nanette Kass Wenger" Women's Heart Program and Women's Heart Center (Belgrade, Serbia) and its collaborative network outline; PHCP – primary healthcare physician; HCW – healthcare workers; SoMe – social media; WHC – Women's Heart Center; DEI – Diversity, Equity and Inclusion; SOD – social determinants of health; RCT – randomized clinical trial

current Minister Ms. Zorana Mihajlović – only being amplified, while aforementioned position still not devised, the Lifestyle Clinic may provide women healthcare workers i.e., doctors, nurses and allied professions, a safe haven to seek medical and self-care advice in collaboration with the existing Safety at Work & Occupational Medicine Department of the University Clinical Center of Serbia.

As any WHC worldwide isn't and shouldn't be a solitary formation lacking interactions, for it can only perpetuate the existing stigma of a "female cult"-like self-sufficient formation, that it genuinely is not, aiming to summarize our proposal for its relationships, we have created the Figure 5.

In a country as Serbia, where allocation of national resources must be highly cost-effective, the WHC is advised to be based in a tertiary University hospital setting and represent an umbrella for all proposed activities.

Besides care offered at the Tertiary level – on both in hospital and face to face clinic-based manner – it should be linked to regional Secondary and Primary healthcare centers via telemedicine [85] that turned to be the "silver lining" of the SARS-CoV2 pandemic and its gaining of momentum should be compliant in forms acceptable for each country and local regulatory settings (including reimbursement options for services rendered).

From an educational perspective, WHC should help bridge the gaps in both core teaching curricula of Schools of Medicine and Nursing, as well as residency and fellowship programs, raising awareness in new generations of healthcare providers on sex-specific diagnostics and therapy beyond the traditional concept.

Research following clinical care provided to women in Primary, Secondary and Tertiary healthcare levels should be adopted. Epidemiological research and registries conducted on all three levels of care, while randomized clinical trials guided by racial and social determinants of health criteria should be designed and conducted at the tertiary level and offered to all interested to participate both nationally and internationally.

Advocacy as a tool for both patients and doctors has been growing worldwide and as such a WHC should offer advocacy tools tailored to needs of any patient within their reach: from in-person guidance by MDs and allied professions on site (Research Nurse are not widespread globally and may take another decade to be utilized more widely), *via* use of simple brochures/pamphlets/themed pad notes or more epidemiological measures-friendly downloading from a website or an app.

Legislate

Legislation pertaining to new modalities of promotion of healthcare and healthy lifestyle is still somewhat vague, as the healthcare providing part of the private sector has flourished immensely over the past two decades and advertising depends mainly on the core ethical values of the owner. Fortunately, among the oldest ones, "VIZIM" primary healthcare physicians' office as the oldest private system of the kind is a good example of appropriate health-related messages both on their walls nation-wide and on their website and both in caring for patients and for their employees.

Currently, global legislative efforts in providing adequate and timely healthcare for women are still meek [2, 86] and although Serbia's laws provides generous paid maternity and paternity leaves, cover ART for couples struggling with infertility and the entire country's population enjoys universal coverage employed or not, access to care due to cultural habits and logistical management, render the fine line between health and social issues, rather blurry, hence hard to help advance healthcare where needed the most.

In Serbia nowadays, the role of progressive, bias-free and equity-promoting experts' groups, East-West Bridge [87] in particular, has been shown effective in working with all stakeholders aiming to implement global initiatives that can also help re-establish Serbia as a leader in region. A country that never knew slavery even in the XIV century (*per Dušan's Code, Constitution written by Serbia's mediaval Emperor Stefan Dušan in 1349*) and formally banned all feudal relationships as such in the XIX century (*the Candlemas Constitution of 1835*), should at least live up to the expectations of its broad-minded ancestors who granted freedom to any slave stepping on medieval Serbia's soil. In that regard, the core team of the "Dr Nanette Kass Wenger" WHC takes special pride in the fact that the initiative has gained multi-partisan support despite all societal complexities where #HeForShe advocacy [88, 89, 90] is usually encountered where you least expect it.

CONCLUSION

Growing popularity of women's cardiovascular and reproductive health, created a flood of reports on outcomes of women derived from protocols designed with other aims, but targeted sex-specific research that endorses substantial changes in local practices and legislation is still pending – not only in Serbia, but worldwide, which emphasizes need for equitable care for all. The concept of WHC's remains sorely needed wherever healthcare provided for women is in any way unsatisfactory, while the proposed Serbian model is adaptable to local, country-specific needs

and should be tailored accordingly. As the "Dr Nanette Kass Wenger" WHC awaits its opening in function of the COVID-19 pandemic that keeps derailing all endeavors, despite stellar home team ones, as long as there remains a will a way shall be made for advancing healthcare of the most fragile.

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Здравље жена у Србији – некад, сада и сутра

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САЖЕТАК

Кардиоваскуларно и репродуктивно здравље жена су нераздвојни кроз историју, међутим, њихова повезаност је била мало проучавана. Тек када су основи њихових веза почели да бивају утврђени, пружање свеобухватног лечења и неге жена даље од „бикини медицине", зависило је од регионалних могућности и степена прогресивног размишљања.

Даља истраживања су идентификовала различите удружености које су различита медицинска стања учинила полноспецифичним, чиме је започет спор, али ипак заокрет у концепцији клиничких студија, што је од посебног значаја имајући у виду да се већина светских кардиоваскуларних препорука базира на резултатима истраживања спроведеним на јако мало броју жена и, још мање процентуално, на женама различитих етничких група.

Тренутно, концепт центара за женско срце зависи од локалних демографских потреба, доступне логистике условљене како финансијском, тако и друштвеном подршком окружења лишеног предрасуда према целом спектруму могућих пацијенткиња: од младих који преиспитују свој родни идентитет, преко жена репродуктивног узраста које покушавају да реализују своју жељу за потомством или су пак здраве, али желе током трудноће да наставе да раде скраћено радно време ако то природа њиховог посла дозвољава, до старијих и оних позног животног доба.

Користећи принцип „Истражи-Образуј-Заложи се-Озакони" као четири стуба напретка кардиоваскуларног лечења и неге жена, настојали смо да пружимо пресек здравственог стања жена у Србији, да представимо текуће пројекте и пружимо предлоге за даља, одржива и изводљива решења за будућност.

Кључне речи: ковид 19; сексуалне разлике; трудноћа; жене у кардиологији; дискриминација; инклузија

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СТРУЧНЕ ВЕСТИ / PROFESSIONAL NEWS

Организовање основне едукације из области трансторакалне ехокардиографије у Србији – становиште Ехокардиографског удружења Србије

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САЖЕТАК
Ехокардиографија је неизоставна дијагностичка вештина кардиолога и лекара других специјалности који учествују у збрињавању болесника са кардиоваскуларним болестима. Овим путем Ехокардиографско удружење Србије саопштава своје ставове у вези са организовањем основне обуке из области трансторакалне ехокардиографије, са циљем уједначавања и подизања квалитета обуке у Србији.
Кључне речи: едукација; ехокардиографија; Србија

УВОД

Широка заступљеност ехокардиографије очекивано је довела до повећаних захтева за едукацијом, а одсуство законских норми у овој области и хетерогеност постојећих едукативних програма доводе у питање квалитет саме обуке и компетенцију која се стиче када се обука заврши.

Ехокардиографско удружење Србије (ЕХОС) већ скоро 20 година окупља водеће ехокардиографисте у Србији са циљем унапређења ехокардиографије кроз организовање и усмеравање едукације из ове области. ЕХОС је практично од свог оснивања званични партнер Европског удружења за ехокардиографију [сада Европског удружења за кардиоваскуларни имиџинг, *European Association of Cardiovascular Imaging* (*EACVI*)] и подржава ставове *EACVI* уз уважавање локалних специфичности [1].

СТАВОВИ И ПРЕПОРУКЕ

Овим путем ЕХОС саопштава ставове у вези са организовањем основне обуке из области трансторакалне ехокардиографије, са циљем уједначавања и подизања квалитета обуке у Србији. Ставови се темеље на препорукама међународних струковних организација и експертским мишљењима [2, 3].

ЕХОС подржава едукативне програме планиране и изведене у складу са доле наведеним ставовима.

1. Трајање практичног дела основне обуке треба да траје најмање три месеца (40 радних сати недељно током 12 недеља), уз менторство самосталног ехокардиографисте у центру који обавља велики број прегледа (минимум 20 на дневном нивоу).
2. Практични део обуке мора да обухвата комбинацију случајева са нормалним и патолошким налазима, са већим уделом патолошких налаза (пожељан однос случајева 20/80% у корист патолошког налаза).
3. Практични део обуке мора да обухвати најчешћа ургентна стања у кардиоваскуларној (КВ) медицини (Табела 1); уколико сва наведена стања не буду заступљена током обуке, треба приказати више архивираних примера и дискутовати их са полазницима.

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Табела 1. Ургентна стања која морају бити обухваћена практичном наставом
Table 1. Cardiovascular emergencies that must be included in practical training

1. Дисекција аорте / Aortic dissection
2. Плућна тромбоемболија / Pulmonary thromboembolism
3. Акутни коронарни синдром / Acute coronary syndrome
4. Тампонада срца / Cardiac tamponade
5. Акутне валвуларне регургитације / Acute valvular regurgitations
6. Акутна дисфункција вештачких валвула / Acute dysfunction of artificial valves

Табела 2. Програм теоријске обуке
Table 2. Program of the theoretical training

Извођење прегледа и подешавање апарата / Performing the examination and setting up the machine
Доплер ехокардиографија: основни принципи / Doppler echocardiography: basic principles
Процена функције леве и десне коморе / Assessment of left and right ventricular function
Акутни инфаркт миокарда / Acute myocardial infarction
Ургентна и стрес ехокардиографија / Emergency and stress echocardiography
Валвуларне мане / Valvular defects
Вештачке валвуле / Artificial valves
Акутни аортни синдром / Acute aortic syndrome
Инфективни ендокардитис / Infective endocarditis
Миокардитис / Myocarditis
Перикардитис и тампонада срца / Pericarditis and cardiac tamponade
Плућна емболија / Pulmonary embolism
Кардиомиопатије / Cardiomyopathies
Интракардијалне масе / Intracardiac masses
Најчешће урођене срчане мане код одраслих / The most common congenital heart defects in adults

4. Теоријска настава мора да обухвати основна знања о анатомији, физиологији и патофизиологији КВ система која су неопходна за разумевање ехокардиографског налаза. Болести и стања које треба обавезно обухватити теоријском наставом сумирана су у Табели 2.
5. Током практичног дела обуке, полазник треба да учествује у прегледу најмање 350 болесника уз надзор ментора и да води дневник урађених прегледа.
6. Обука треба обавезно да укључи следеће: М-мод, дводимензионалну ехокардиографију, доплер у боји, пулсни, континуирани и ткивни доплер. Пожељно је да полазник буде упознат са основама тродимензионалне, стрес, контрастне и трансезофагеалне ехокардиографије и принципима анализе деформације миокарда, без обавезе да полазници након тромесечне обуке самостално изводе ове ехокардиографске модалитете.
7. Након завршеног практичног и теоријског дела обуке, кандидат треба да приступи завршном испиту, уз претходну потврду супервизора да је овладао техником извођења прегледа. Уколико непосредни супервизор сматра да кандидат није овладао техником прегледа, практични део обуке треба продужити до постизања потребног нивоа обучености.
8. Испит треба да се састоји од питања са понуђеним одговорима и видео-питања, тј. препознавања КВ патологије на практичним примерима.
9. Кандидати који тачно одговоре на више од 60% питања положили су завршни испит и стекли право на издавање сертификата о завршеној обуци.
10. Након завршене обуке, полазници треба да стекну способност и знање да обављају трансторакалне ехокардиографске прегледе под надзором самосталног ехокардиографисте, до стицања искуства за самостално, ненадгледано извођење ових прегледа.
11. Потврда о завршеној обуци није еквивалент лиценце за самосталан рад и нема такву правну снагу. Лиценцирање, тј. издавање дозволе за самостално извођење ехокардиографског прегледа је у надлежности законом предвиђених органа и/или лица у здравственој установи у којој лекар са завршеном обуком обавља лекарску праксу.
12. Иако сва лица са завршеним студијама медицине могу похађати ехокардиографску обуку, самостално извођење прегледа након завршене едукације је ограничено на лекаре који према прописима Републике Србије имају формално право да самостално изводе, интерпретирају и потписују ову врсту прегледа (по правилу специјалисти и супспецијалисти одговарајућих профила).

Едукацију из ове области која не испуњава наведене критеријуме ЕХОС не сматра ехокардиографском обуком која оспособљава кандидата за квалитетно извођење трансторакалних прегледа.

ЗАКЉУЧАК

Анкета ЕХОС-а о стању ехокардиографије у Србији открила је потребу за унапређењем извођења ехокардиографских прегледа[3]; у истој анкети само 12% чланова удружења који су учествовали у анкети било је потпуно задовољно достигнутим нивоом едукације. Поред тога, анкета ЕХОС-а спроведена током пандемије ковида 19 указала је на значај познавања базичног ултразвука плућа, те овај вид едукације треба обухватити ургентном ехокардиографијом [4, 5]. Ови подаци указују на велику потребу за едукативним програмима високог квалитета у Србији. С тим у вези, ЕХОС предлаже наведени оквир као полазну основу за унапређење квалитета едукације, до тренутка уређивања ове важне области одговарајућим законским актима и прописима.

Потенцијални сукоб интереса: Иван Станковић, Милица Деклева-Манојловић, Марина Дељанин-Илић, Димитра Калимановска-Оштрић и Александар Н. Нешковић су учествовали у извођењу ехокардиографске обуке под окриљем ЕХОС-а. Данијела

Трифуновић-Замаклар, Босиљка Вујисић-Тешић и Димитра Калимановска-Оштрић су учествовале у извођењу обуке под окриљем Универзитетског клиничког центра Србије. Љиљана Јововић је била оснивач и руководилац Ехокардиографске школе у Институту за кардиоваскуларне болести „Дедиње“ у периоду од 2007. до 2018. године. Анастазија Стојшић-Милосављевић је учествовала у извођењу обуке под окриљем Института за кардиоваскуларне болести Војводине. Зорица Младеновић и Слободан Обрадовић су учествовали у извођењу обуке под окриљем Војномедицинске академије. Аутори негирају постојање других потенцијалних сукоба интереса.

Potential conflict of interest: Ivan Stanković, Milica Dekleva-Manojlović, Marina Deljanin-Ilić, Dimitra Kalimanovska-Oštrić, and Aleksandar N. Nešković participated in performing echocardiographic training under the auspices of the Echocardiographic Society of Serbia. Danijela Trifunović-Zamaklar, Bosiljka Vujisić-Tešić and Dimitra Kalimanovska-Oštrić participated in the training under the auspices of the University Clinical Center of Serbia. Ljiljana Jovović was the founder and the head of the Echocardiography School at the Dedinje Institute for Cardiovascular Diseases during the 2007–2018 period. Anastazija Stojšić-Milosavljević participated in the training under the auspices of the Institute for Cardiovascular Diseases of Vojvodina. Zorica Mladenović and Slobodan Obradović participated in the training under the auspices of the Military Medical Academy. The authors deny other potential conflicts of interest.

Финансирање: Трошкове објављивања рукописа сноси Ехокардиографско удружење Србије.

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Organization of basic education in transthoracic echocardiography in Serbia – a viewpoint of the Echocardiographic Society of Serbia

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SUMMARY

Echocardiography is an indispensable diagnostic tool of cardiologists and other specialties involved in proving care to cardiovascular patients. In this paper, Echocardiographic Society of Serbia provides its viewpoint regarding the organization of basic education in transthoracic echocardiography, aiming at homogeneity of education and improving the quality of echocardiographic training in Serbia.

Keywords: education; echocardiography; Serbia

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LETTER TO THE EDITOR / ПИСМО УРЕДНИКУ

Spontaneous splenic rupture in marginal zone lymphoma at presentation

Dear Editor,

Spontaneous (atraumatic) splenic rupture (SSR) may occur even in a normal healthy spleen; however, in most cases, it happens in an enlarged spleen due to serious infections, malignant diseases before and after therapy, metabolic diseases, vascular, and hematological diseases. Generally, it is always a life-threatening condition that requires an immediate diagnosis and splenectomy. To the best of our knowledge, SSR has been rarely described in lymphoma of the marginal zone although these patients always have significant splenomegaly [1, 2, 3]. We present a patient that could be the third case ever reported.

A 57-year-old male with complaints of epigastric pain radiating towards the left hemithorax and towards the left shoulder, which appeared seven days earlier, was admitted at the surgical department. There was no history of trauma. Two years previously, he had been submitted to laparoscopic cholecystectomy, after which he was told about having a moderately enlarged spleen. Being symptom-free, he ignored the information until recently, when pancytopenia and hepatosplenomegaly were discovered during a routine checkup.

At admission, he was pale but hemodynamically stable, with a moderately enlarged abdomen, epigastric tenderness, hepatosplenomegaly, and signs of free fluid within the abdomen. Laboratory data was as follows: hemoglobin 76 g/l, hematocrit 22%, platelets 90×10^9/l, white blood cells 1.7×10^9/l (differential leukocyte formula: neutrophils 77%, lymphocytes 13%, monocytes 6%, eosinophils 3%, basophils 1%), lactate dehydrogenase 740 U/l (normal range 220–460 U/l), glycaemia 8.4 mol/l, total proteins 53 g/l (albumin 35 g/l), C-reactive protein 112.7 mg/l (normal range 0–5 mg/l). The rest of biochemistry and clotting screen tests were within normal limits. Bone marrow aspirate was normal.

Multidetector computed tomography (MDCT) showed a mild bilateral pleural effusion, enlarged liver (22 cm), enlarged spleen (25 cm) with signs of rupture at the upper pole and several hypodense zones probably caused by infarctions, as well as free fluid of higher density corresponding serohemorrhagic content (Figure 1).

Left subcostal laparotomy was done, through which serohemorrhagic fluid was aspirated, a 4.5-cm rupture of the upper pole of the spleen was found, and splenectomy and biopsy of the liver were performed. The intraabdominal lymph nodes were not enlarged.

The spleen was 280 × 180 × 120 mm in diameter, weighing 2700 g, firm and with a ruptured upper pole. On cross section it showed a large homogenous granular structure. Histology was significant for congestion of the red pulp and proliferation of the white pulp, showing merging and spreading of diffuse infiltrates into the surrounding red pulp. The infiltrate was composed of small and medium sized cells of lymphoid and monocytic morphology corresponding with lymphoproliferation in non-Hodgkin lymphoma (NHL). The area of rupture in the upper pole corresponded with necrosis and fresh infarction. Histology of the liver showed small cell infiltration of portal spaces. Immunohistochemistry showed that the malignant cells were CD20+, CD79a+, CD3-, CD5-, bcl-2 -/+, CD10-, CD23-, cyclin D1-, CD38-, CD138-, kappa-, lambda-, DBA+/-, VD25-, CD123-, CD34-, CD8-, CD30-, CD15-, CD61-, TRAP-, and Ki67 positive in about 10% of cells. Histology and immunohistochemistry were consistent with the diagnosis of splenic marginal zone lymphoma (SMZL). Histology of the liver showed small lymphoid cell infiltration within portal spaces with the same histological and immunophenotypic characteristics as those seen in the spleen. Bone marrow aspirate and histology was free of lymphoma. Virology was negative. The final diagnosis of SMZL with liver involvement was established.

The postoperative recovery was uneventful. High number of platelets of 1020×10^9/l required treatment with low-molecular-weight heparin and aspirin. The post-splenectomy prophylactic vaccination was performed.

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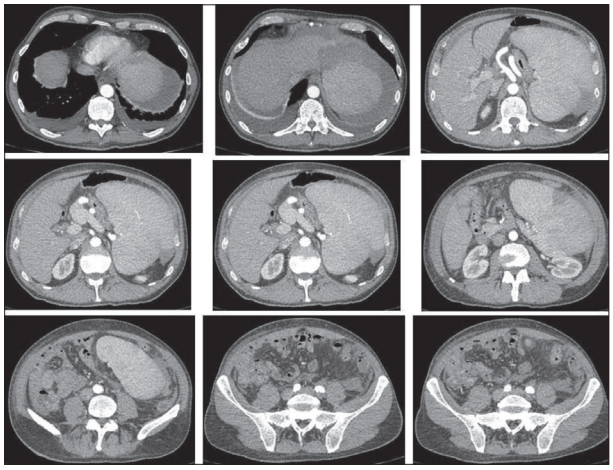

Figure 1. Multidetector computed tomography showing mild pleural effusion, enlarged liver (22 cm), enlarged spleen (25 cm) with signs of rupture in the upper pole and a number of hypodense zones probably caused by infarctions; there is a certain amount of free fluid of higher density corresponding serohemorrhagic content

Although splenectomy has been regarded as a treatment of choice of SMZL, due to liver involvement, additional immunochemotherapy will be applied.

SMZL appears in less than 2% of NHL. Histologically, it shows the proliferation of mature B-lymphocytes. There are three forms of SMZL: nodal MZL, splenic MZL, and extranodal MZL of mucosa-associated tissue. The diagnosis is based on the localization of the disease, histology, and immunohistochemistry. Tumor cells are CD20+, CD79a+, CD5-, CD10-, CD23-, CD43-, and Cyclin D1-. Most frequently they have an indolent course even in patients with bone marrow involvement. If the disease is localized in the spleen only, splenectomy is necessary to establish the diagnosis. In such cases splenectomy is the only treatment requiring no further treatment, but close follow-up is necessary. Rupture of the spleen as in the presented case may be the result of significant enlargement of the spleen due to infiltration of the splenic tissue by the tumorous tissue and infarction in the subcapsular space. Smaller size infarctions may be found in the other areas of the spleen, which may be seen on MDCT, as in our patient.

SSR may have an acute or subacute clinical course, as in the presented case. Thus, clinical pictures of SSR may vary depending on the rupture size, as well as on possible adhesions around the spleen preventing major bleeding in a short period of time. Typically, it is followed by abdominal pain radiating toward the left hemithorax and the left shoulder, as seen in our patient, nausea, vomiting, and hemodynamic changes [4, 5]. In rare cases, pain may be absent, so that possible rupture should be considered if patients with splenomegaly develop a hemodynamic change. In such cases, abdominal ultrasonography and computed tomography are to be performed [6, 7]. In unclear cases, a diagnostic lavage of the peritoneal cavity may be recommended [8]. If the rupture is a major one, serious bleeding can occur and requires urgent diagnosis and surgery; otherwise, it may have a fatal outcome [5, 9, 10]. It is important to know that there is no laboratory or imaging method to predict a rupture, so it is important to take the possibility into account.

Three explanations are offered to clarify the nature of splenic rupture in lymphomas. Parenchyma of the spleen in hematological malignancies becomes infiltrated with malignant cells, which leads to an increase in the intrasplenic pressure, which in turn transmits onto the splenic capsule, causing rupture. The disturbances of coagulation or thrombocytopenia may cause intrasplenic or subcapsular bleeding. Finally, an infarction that leads to rupture may be caused by increased pressure of the proliferated tissue on intrasplenic blood vessels [3, 4, 8, 11].

There are several pathologies that may lead to SSR. In an analysis of 632 publications presenting with SSR, in 845 patients within a period 1980–2008, neoplasms were the cause of SSR in 30.3%, infections (malaria, infectious mononucleosis) in 27.3%, inflammatory non-infectious diseases (autoimmune diseases, hemolytic anemia) in 20%, rupture after different chemotherapeutic agents in 9.2%, mechanical rupture in 6.8%, and idiopathic rupture of the normal spleen in 7% [10, 11].

Several hematological diseases result in splenomegaly. However, SSR in those diseases is an unusual event. Among them, SSR is most frequent in NHL. In a series of 136 published cases of SSR in a period 1861–1995, SSR in NHL was registered in 34%, in acute myeloid leukemia in 34%, and chronic myeloid leukemia in 18% [8, 11]. Among NHL cases, spontaneous rupture appears with similar frequency in diffuse large B-cells lymphomas, blast variant of mantle cell lymphoma, anaplastic large cells, and hepatosplenic lymphoma, and is rarely described in diffuse histiocytic lymphoma, follicular lymphoma, malignant lymphomonocytic, and diffuse histiocytic lymphoma [3, 12]. In this series, no single case of SSR was registered in patients suffering from SMZL lymphoma.

Conflict of interest: None declared.

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Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови**,** наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

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

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

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

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

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

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

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

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

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

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

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

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

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

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

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

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

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

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

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

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

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

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

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
Српско лекарско друштво
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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

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ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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