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In more than 100 countries around the world, the World Kidney Day is celebrated every other Thursday in March, starting from 2006 on the initiative of the International Society of Nephrology and the International Federation of Kidney Foundations.

The goal is to raise awareness about the importance of the kidney, an organ that plays a key role in maintaining life, and to inform the public that although kidney disease is frequent and very serious it can be prevented and treated successfully. The theme of this year's World Kidney Day "Healthy kidneys to everyone and everywhere" aims to highlight the fact that the provision of high quality health services for the protection and promotion of the kidney health must be universal and equal, with a constant reduction of inequalities regarding the various racial and socioeconomic population groups and their access to health care.

Svetski dan bubrega se obeležava svakog drugog četvrtka u martu, od 2006. godine, na inicijativu Međunarodnog društva za nefrologiju i Međunarodne federacije fondacija za bubreg u više od 100 zemalja širom sveta, sa ciljem podizanja svesti o važnosti bubrega u održanju života i informisanja javnosti o tome da su bolesti bubrega česte, veoma ozbiljne, ali da ih je moguće prevenirati i uspešno lečiti.

Slogan ovogodišnjeg Svetskog dana bubrega „Zdravi bubrezi svima i svuda” ima za cilj da istakne činjenicu da pružanje visokokvalitetnih zdravstvenih usluga za zaštitu i unapređenje zdravlja bubrega mora biti univerzalno i ravnopravno, sa smanjenjem nejednakosti u dostupnosti zdravstvenoj zaštiti među različitim rasnim i socioekonomskim grupama stanovništva.



## Dental arch monitoring by splines fitting error during orthodontic treatment using 3D digital models

Praćenje oblika zubnog luka odstupanjima fitovanih splajnova tokom ortodontske terapije primenom 3D digitalnih modela

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### Abstract

**Background/Aim.** Researchers in the field of dentistry have been conducting research into modelling and defining dental arches equitations. Nowadays, when 3D digital modelling is commonly utilized in dentistry, the approach to modelling, analysis and synthesis has changed. Clinical researches are related to aesthetic and functional analysis. The aim of this study was to increase repeatability and accuracy of defining and determining the coordinate system of the jaw as well as to defining mathematical criteria for monitoring and evaluating orthodontic treatment. **Methods.** In this study, we used the plaster models of the jaw, optical scanner with structured light, 3D digital models, computer aided design (CAD) engineering tools adjusting the coordinate system, spline fitting of 3rd, 4th, 5th, 6th, 7th and 8th degrees. **Results.** Splines of 3rd, 4th, 5th, 6th, 7th and 8th degrees were fitted from the initial state (K0) in all 10 successive controls (K1, K2, K3, ..., K10). All splines were fitted through 12 points, from the right to the left side of the jaw: 6-5-4-3-2-1-1-2-3-4-5-6. Tabular and graphic presentations of the maximum and average deviation of dental arch fitting curves in successive controls were given. **Conclusion.** The parameters of the maximum and average errors of fitting curves converge the dental arch values that are lower than the accuracy of the used optical scanners. The average error of fitting provides a general picture of the entire dental arch at each stage of treatment. Maximum error fitting points at a specified tooth where the largest deviation.

### Key words:

computer-aided design; dental arch; jaw; malocclusion; orthodontic appliance design.

### Apstrakt

**Uvod/Cilj.** Istraživači u oblasti stomatologije, posebno kliničari, već dugo se bave istraživanjima koja se odnose na modeliranje i definisanje oblika i parametara zubnog luka. Danas, kada je 3D digitalno modeliranje postalo uobičajena praksa u stomatologiji, promenio se i prilaz modeliranju, analizi i sintezi u ortodontici. Klinička istraživanja oblika zubnog luka direktno se odnose na estetsku i funkcionalnu analizu zubnog niza (nivelacija, okluzija, zagrižaj). Cilj rada bio je da se poveća ponovljivost i preciznost definisanja i određivanja koordinatnog sistema vilice i definišu matematički kriterijumi za praćenje i ocenjivanje ortodontske terapije. **Metode.** U radu su korišćeni gipsani modeli vilice, optički skener sa strukturisanom svetlošću, 3D digitalni modeli vilice i *Computer Aided Design* (CAD) i inženjerski alati. Sprovedeno je podešavanje koordinatnog sistema i fitovanje splajnova trećeg, četvrtog, petog, šestog, sedmog i osmog stepena. **Rezultati.** Splajnovi (trećeg, četvrtog, petog, šestog, sedmog i osmog stepena) fitovani su u odnosu na početno stanje (K0), za svih 10 uzastopnih kolona (K1, K2, K3, ..., K10). Svi splajnovi su fitovani u 12 tačaka, sa leve i desne strane vilice: 6-5-4-3-2-1-1-2-3-4-5-6. Dat je tabelarni i grafički prikaz maksimalnih i prosečnih odstupanja fitovanih krivih linija dentalnog luka u sukcesivnim kontrolama. **Zaključak.** Parametri maksimalne i prosečne greške fitovanja krivih linija dentalnog luka konvergiraju vrednostima koje su manje od tačnosti korišćenih optičkih skenera. Prosečna greška fitovanja daje opštu sliku celokupnog dentalnog luka u svakoj od faza terapije. Maksimalna greška fitovanja ukazuje na tačno određeni zub gde su odstupanja najveća.

### Ključne reči:

kompjuterski podržan dizajn; zubni luk; malokluzija; ortodontski aparati, dizajn.

### Introduction

3D digital modelling is widely used in orthodontics today<sup>1,2</sup> for the following: laser scanning and computer

aided design (CAD) modelling of complex shapes and surfaces, teeth and jaws, as a separate area of engineering modelling, analysis and synthesis of orthodontic parameters in 3D digital and plaster models (precision, repeatability, validity,

reliability), occlusion analysis, planning and monitoring orthodontic treatment and analysis and synthesis of dental arch shape.

A specific example of research<sup>3</sup> involves comparisons of the dental arch shape for its axis in relation to its crown and root end, of classes 1 and 2, of the group 1 of malocclusion, using WALA points<sup>4</sup> and by WALA points, orthodontic treatment can be successfully planned.

The measurement and analysis of 20 models (virtual, plaster) was done with the following parameters: mesiodistal tooth width, intercanine and intermolar distance, as well as the width of the arch<sup>5</sup>. Nonparametric statistics showed that, from the aspect of precision and repeatability of results, the utilization of the OrtCAD was greater than in conventional measurements on plaster models.

The analysis of the ideal dental arch shape, showed a fourth degree polynomial, and in case of patients missing a tooth (particularly from the first molar to the central incisor),  $\beta$  function represented a better solution for the simulation of dental arch shape<sup>6</sup>.

A research by Park et al.<sup>7</sup> provided results regarding the new classification of dental arch shapes in an ideal bite, obtained by 3D digital models and the cluster analysis (divided into 4 clusters, based on the orthodontic parameters) of the classification of dental arch shapes with distances: between canines, premolars and molars.

The study by Slaj et al.<sup>8</sup> showed the dental arch shapes obtained by the factor analysis of 137 digital models. The key element of factor analysis was the ratio between the lower canine width/height, which comprises 82.8% of variability ( $p < 0.001$ ). The dental arch shape in the lower jaw was substantially different in posterior teeth, whilst in the upper jaw the greatest changes were observed in anterior teeth.

A combined method of determining a dental arch by superimposition in the lower jaw, using two techniques – cone beam computed tomography (CBCT) and 3D modeling was shown in the study by Park et al.<sup>9</sup>. The results of the research showed that 3D modelling is a simpler and more reliable means for calculating the length of the dental arch.

A comparison of precision of 3-dimensional models was obtained by an intraoral scanner, 3D digital model and conventional measuring<sup>10</sup>. The obtained dental arch length for the 3D models was: precision –  $1.6 \pm 0.6 \mu\text{m}$  and validity –  $5.3 \pm 1.1 \mu\text{m}$ . The same parameters for intraoral scanner were  $12.5 \pm 2.5 \mu\text{m}$  and  $20.4 \pm 2.2 \mu\text{m}$ , and for conventional measuring they are  $32.4 \pm 9.6 \mu\text{m}$  and  $58.6 \pm 15.8 \mu\text{m}$ . In all these analyses  $p < 0.001$  was the same.

Finally, the digital models provide reliable and clinically dependable orthodontic parameter results for the tooth width, dental arch circumferences, intercanine and intermolar widths<sup>11</sup>. The conclusion is that the digital model produces a lower degree of variation in the measured results compared to the conventional measuring method in the Little's Irregularity Index.

The research by Kook et al.<sup>12</sup> dealt the measurements on the 3D models and plaster impressions of 7 dental arch parameters (width/distance of the first 6 maxillary teeth, left

and right) in a sample of 27 patients. The overlapping parameter showed significant statistical differences, which confirmed the impact of brackets on the 3D models. The conclusion is that the overlapping parameter should be carefully considered and measured in future while paying a special attention to the position of each individual tooth.

AlHarbi et al.<sup>13</sup> suggested that, in order to ideally describe the dental arch curve, a polynomial of a higher degree had to be used. This curve should possess a significant flexibility, so it can be adjusted to suit any dental arch size and had to include the jaw asymmetries, if any. Starting from the fact that a dental arch is an imaginary curve, the descriptive information, i.e., function represents a set of discrete points. For that reason, a mathematically obtained curve should be adjusted to the individual points<sup>14, 15</sup>. This approach refers to the curve adjustment or interpolation. Curve adjustment is a concept used for adapting the mathematical shape of a curve to the curve of the actual dental arch shape. The curve generated by proposed mathematical functions is adjusted in accordance with marks on the teeth believed to precisely define the dental arch. With the aim of discovering the optimal mathematical function to describe the dental arch curves, a number of authors tested various mathematical functions (models) which could best serve this purpose. Some of these models are: conic curve shapes, U-curve, cubic equations, equations from 2nd to 8th degree, mixed models and the beta function<sup>16, 17</sup>. Finally, it is difficult to generalize their findings or draw conclusions for a number of reasons: different objectives, different study samples with different criteria and different methodology.

This analysis helps us conclude that the analysis and synthesis of dental arch are elements of overall orthodontic treatment. However, all researches investigate groups of patients, whereby the general or common characteristics can be determined.

The previous analysed studies<sup>18–20</sup> did not consider adjusting the coordinate system of the jaw. Probably the reason is the American Board of Orthodontics (ABO)<sup>18</sup> recommendations published for the first time in 2013 and the latest version was published in 2016. Comparisons of dental arch curves is not possible if the coordinate system is not defined uniquely and the repeatability is not provided in all successive controls.

The first objective of this study was analysis and improvement of the ABO recommendations. The second objective was the definition of mathematical criteria for monitoring and evaluation of orthodontic treatment. For this purpose, the maximum and average fitting errors of dental arch curves, interpolating splines of 3rd, 4th, 5th, 6th, 7th, and 8th degrees will be monitored.

In existing literature, there is no extensive mathematical analysis of the dental arch shape in persons with normal occlusion in the course of orthodontic treatment. Therefore, the aim of this study was also to define the dental arch function as a polynomial, from 3rd to 8th degree, and to perform its thorough analysis following all the stages of orthodontic treatment. The core of the problem is that during the treatment, the position of teeth in the jaw changes, which leads to a

change in the position of coordinate system of the jaw, based on which the function of dental arch is modelled. The consequence of this is that the shapes of the dental arch for two conditions – stages of treatment – can be compared with a relatively low precision. As a result, here we established a global coordinate system of the jaw (GCSJ) following the advanced method of ABO, thereby eliminating this negative impact, so that the dental arches can be absolutely accurately compared at each individual stage of orthodontic treatment. This research represents a part of the overall model for monitoring and analysis of orthodontic treatment, from the aspect of teeth alignment, using the 3D digital models<sup>1</sup>.

## Methods

This study includes scans of 22 impressions, obtained from the patients' archives at the Clinic for Jaw Orthopaedics, Faculty of Dentistry in Belgrade, using the ATOS scanner<sup>21</sup>, with the precision lower than 10  $\mu\text{m}$ . The impressions were randomly chosen. The only criterion was normal occlusion. 3D modelling was performed by the Siemens NX10 software<sup>22</sup>.

The curved lines describe the dental arch in normal occlusion, meaning it is in X-Y (occlusal) plane in the orthodontic coordinate system of the jaw digital model, called GCSJ<sup>1</sup>. It is defined by ABO rules<sup>18</sup>, which state digital model requirements and these instructions represent a commonly accepted *de facto* world standard for digital models of jaws. In accordance with the guidelines, the digital models were generated in the PLY, STL or OBJ format files, from which further analyses were performed. In addition, the scanners used for generating the 3D digital models need to have resolution of 0.10 mm or more and precision<sup>18</sup> of at least 0.20 mm. One of the basic characteristics of the procedure for determining GCSJ is that it provides the repeatability of measuring (scanning) one jaw model on different scanners, which is extremely significant for the 3D digital models. On the other hand, when comparing several different jaw models, which occurs in the course of an individual patient's orthodontic treatment, the ABO guidelines do not provide enough precision. This is mostly related to defining Y-axis orientation (Y-Z: median plane) and zero – the origin of coordinate system. This paper improved the ABO guidelines, thereby also enhanced the results of our research.

### Digital model orientation and coordinate system definition

The ABO defines requirements for digital models (scan resolution and accuracy, measuring units, file formats and mesh topology) and the procedure for adjusting the coordinate system<sup>18</sup>.

Defined procedure, in essence, is very similar to the procedures used by engineers in coordinate metrology. This is quite logical, because this is completely an engineering problem. The jaw coordinate system adjusting procedure is a typical "3-2-1" procedure. Numbers "3-2-1" in the procedure name define degrees of freedom that are fixed in each of the

three steps. To define the coordinate system exactly, it is necessary to fix all 6 degrees of freedom (3 rotations and 3 translations) and how much of each solid body decoupled<sup>19</sup>. ABO "3-2-1" procedure is related to: XY plane – occlusal: fitting a plane using the least squares method through 16 points (molars and premolars cusp tips); Y axis orientations, mid-sagittal: through the mid-points of line segments between 2 left +2 right points located in the mid-palatal raphe; Origin (0,0,0): at a point that lies approximately half-way between the most anterior and most posterior teeth.

The ABO procedure has two major drawbacks: it is not possible to provide a satisfactory repeatability of Y axis orientation in successive controls of a patient jaw; the origin position is not precisely defined. During the treatment the incisors are the most shifted teeth; their intake for reference is completely wrong. The ABO procedure uses the term "approximately" for origin position. "Approximately" it is not good enough for accurate measurement, especially in the case of dental arch creation and their comparison in successive controls.

The ABO procedure provides repeatability when one unchanged jaw model is scanned<sup>18</sup> by using different scanners. This is not the case when monitoring the entire orthodontic treatment. To be able to compare the scanned models of two successive check-ups, it is essential that the coordinate systems is always in the same position. It is an axiom of coordinate metrology. If we want to compare the equations of dental arch, as it was done in this paper, adjusting the coordinate system is the most important step in the entire procedure. Mistakes made in the initial step inevitably lead to wrong conclusions. The erroneous coordinate system settings have the greatest impact on the measurement error<sup>19</sup>.

The ABO procedure described deficiencies; they are enhanced by the new determination of the Y axis direction, and more accurate determination of the origin. Figure 1 shows these improvements.

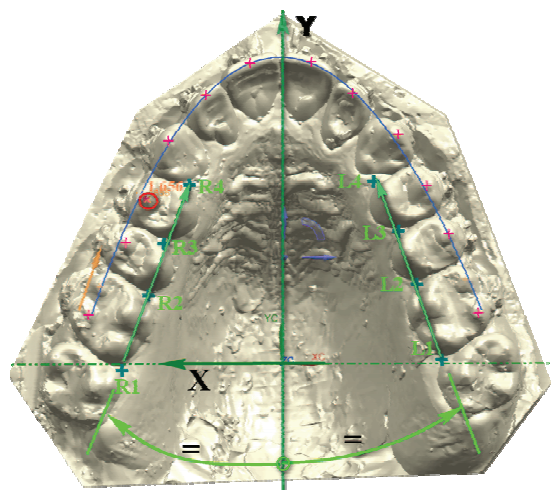


Fig. 1 – Jaw coordinate system definition.

Y axis is the bisector of two lines on the left and right side of the jaw (Figure 1). Each line is interpolated through 4 points using the least squares method. On the right side these interpolating points are marked in Figure 1 in the following

way:  $R_1$ - $R_2$ - $R_3$ - $R_4$ . On the left side these interpolating points are marked in Figure 1:  $L_1$ - $L_2$ - $L_3$ - $L_4$ . Pairs of points ( $R_1$ - $L_1$  and  $R_4$ - $L_4$ ) were determined in the same way as defined in the ABO guide<sup>18</sup>. Additional pairs of points  $R_2$ - $L_2$  are defined in a similar way and they are located between 4th and 5th tooth on the right and left side, respectively. Additional pairs of points  $R_3$ - $L_3$  are located between 5th and 6th tooth on the right and left side, respectively. A new improved method uses 8 points ( $4 + 4$ ) for the Y axis definition; the ABO guide uses only 4 points ( $2 + 2$ ) for the Y axis definition. A larger number of points improve repeatability of the Y axis settings.

Coordinate system origin is fixed as the intersection between previously determined Y axis and temporary auxiliary line defined by the points  $R_1$  and  $L_1$ , as shown in Figure 1. Intersection point defined the origin (0, 0, 0). X axis is perpendicular to Y axis, and its direction is defined by the right-hand rule. X axis, as expected, does not pass through points  $R_1$  and  $L_1$ . The human body is never perfectly symmetrical.

The origin is determined by the position of the molars, which cannot be displaced (or very minimally). The incisors are the most displaced teeth during orthodontic treatment and should not be taken as a reference for coordinate system origin.

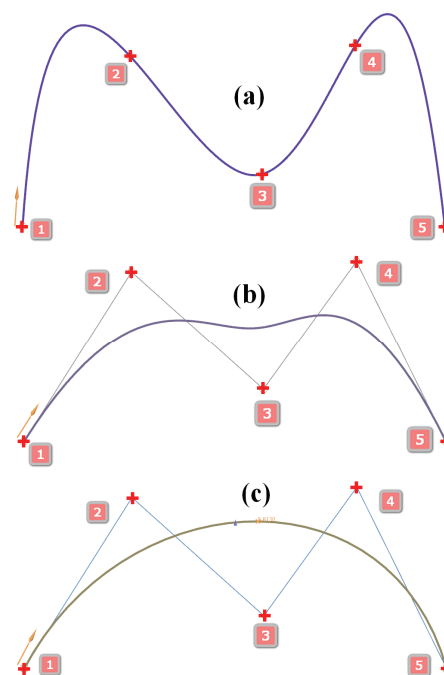
In this paper, interpolation (fitting) curves over all digital models of the upper and lower jaw were made over the same set of digital models presented in the study by Majstorović<sup>1</sup>, as already mentioned.

For the fitting curves which define the dental arch we used the Computer Aided Design, Computer Aided Manufacturing, Computer Aided Engineering (CAD/CAM/CAE) system by world renowned medical equipment manufacturer – Siemens. The software Siemens NX10 is used for the general purpose<sup>22</sup>; it is primarily intended for applications in mechanical engineering. Its flexibility and openness of the architecture allows it to be easily applied in very different areas. Its advantage over the specialised software packages in the area of orthodontics is its dedication to sophisticated modules for the complex spatial forms (free-form, sculptured surfaces).

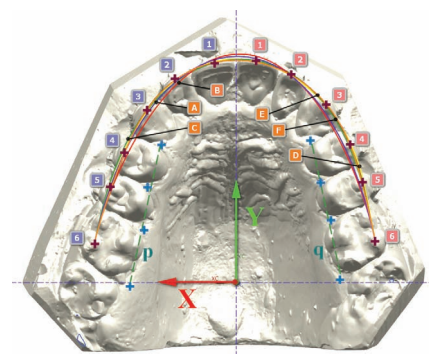
All splines created with Siemens NX10 are<sup>22</sup> the Non Uniform Rational B-Splines (NURBS). The literature providing a mathematical foundation<sup>20</sup> is widespread and easily accessible. Splines are widely used, not only in various engineering areas. Their utilisation in the CAD/CAM/CAE systems is simple and intuitive. There is no modern CAD/CAM/CAE system that does not include the ability to create splines. In this section, the terms “B-Spline” and “Spline” are used interchangeably. There are three creation methods for splines (Figure 2): by poles – causes the spline to gravitate towards each data point (that is, pole), poles form a control polygon which determines the shape of spline; through points: the spline passes through a set of data points; fit: the spline does not necessarily pass through the points, except at the endpoints.

In this study, splines were fitted through the points of digital models, which are located at the tips of the teeth

(from the right to left side): 6-5-4-3-2-1-1-2-3-4-5-6; marked in Figure 3. A total of 12 points are used for all the interpolations. As shown in Figure 1, interpolated curve does not pass through the points on the digital model. Some points are closer to the curve, others are away from it. Siemens NX10 marks the most distant point from the fitted curve. In the example in Figure 1, the point with maximum deviation is marked with a small red circle. Siemens NX10 shows the value of the maximum deviation; Figure 1 shows that this value is 1.656 mm on the fourth tooth on the right side. This is a clear indicator for the dentist which tooth to pay more attention to. In addition, Siemens NX10 gives the value of the average deviation of all points on the fitted curve. The user has information on the average deviation, maximum deviation value and the location of maximum deviation.



**Fig. 2 – Spline interpolation methods: a) through points, b) by poles, c) Fit.**



**Fig. 3 – Dental Arch Fitted splines.**

**Label A: 3rd (purple); Label B: 4th (red); Label C: 5th (green); Label D: 6th (yellow); Label E: 7th (orange); Label F: 8th (brown) degrees.**

Another important aspect is the degree of the fitted spline. Every spline has a degree – a mathematical concept

referring to the degree of the polynomial that defines the curve. The degree is generally one less than the number of points in a spline segment<sup>22</sup>. For this reason, it is not possible to have a spline with fewer points than the degree. A higher degree curve is stiffer in the sense that its poles have to be moved a long way to produce any appreciable change in the shape of the curve. Lower degree curves are more pliable, and tend to follow their poles much more closely.

Figure 3 adequately illustrates the problem of selecting the degree of fitted curve of dental arch. The figure shows the interpolated splines from 3rd to 8th degrees.

Zero or a very small value (smaller than the accuracy of the scanner) of average and maximum deviation of the fitted spline for dental arch is a clear indicator that the treatment is reaching its completion.

Thanks to the computer speed and implemented algorithms, several spline fittings can be instantly executed. The user (dentist) interpolates splines of 3rd, 4th, 5th, 6th, 7th and 8th degrees in all stages of orthodontic treatment. This activity can be carried out automatically and does not require any additional knowledge or training.

The convergence of the maximum and average deviation of the fitted splines are the criteria for selecting appropriate curve's degree. The curves whose degree is not suitable will not converge but diverge.

A key requirement is the constancy of the position of the coordinate system. If this condition is not fulfilled, the comparisons are not possible. As already pointed out, the adjustment of the coordinate system is a critical step. Errors made in the initial step lead to wrong conclusions. Therefore, a special attention needs to be paid to a problem of the coordinate system adjustment.

## Results

The developed method was applied on a patient undergoing orthodontic treatment. In each of the stages of the treatment an impression was taken on the basis of which a plaster model was created. All plaster models were scanned

using optical scanners<sup>23</sup> in the Standard Tessellation Language (STL format – stereolithography). The obtained digital models were transferred onto engineering software<sup>22</sup> where they served as a basis for the application of previously described method.

The original state (without the fitted braces) was marked as "K0". Each of 10 check-ups was performed in an interval of one month (of wearing braces).

Splines (3rd, 4th, 5th, 6th, 7th and 8th degrees) are fitted from the initial state (K0) in all 10 successive controls (K1, K2, K3, ..., K10). All splines were fitted through 12 points, from the right to the left side of the jaw: 6-5-4-3-2-1-1-2-3-4-5-6. The points for interpolation are marked with red crosses in Figure 3. Each interpolated spline at its beginning has a slope of line "p" and at its end has a slope of line "q", as shown in Figure 3. The bisector line "p" and "q" determines the Y axis direction as explained in the chapter "Adjusting the coordinate system of the jaw".

For each fitted spline Siemens NX10 gives to the user the following information set: value of the Average Fitting Error, value of the Maximum Fitting Error and indicates with an asterisk which points have the maximum value of fitting error. The values obtained for all 11 digital models are presented in Table 1. Column "Deg." indicates the degrees of fitted splines. Column "Err." is divided into two rows marked "M" and "A" for each degree of interpolated splines. "M" stands for Maximum Fitting Error and "A" stands for Average Fitting Error. Columns "K0, K1, K2, K3, K4, K5, K6, K7, K8, K9, K10" indicate all stages of the orthodontics treatment.

The values from Table 1 are shown in the diagrams. The diagram of the maximum deviations for all check-ups is shown in Figure 4. The diagram of the average deviations for all check-ups is shown in Figure 5. The horizontal axis represents the stage of the orthodontic treatment. The vertical axis shows the deviation in mm. Each degree of fitted splines is displayed in a different colour and marked with different graphic symbol (legend of symbols is shown in the diagrams).

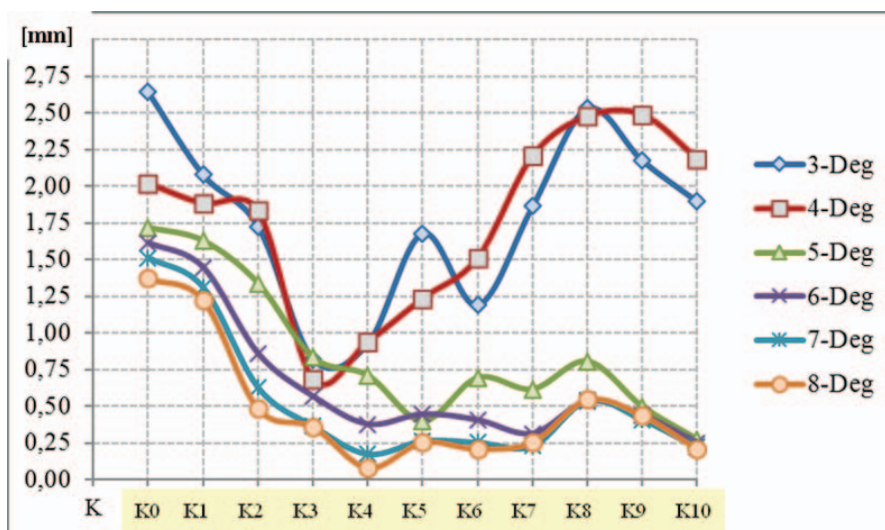


Fig. 4 – Maximum error fitting deviation diagram.

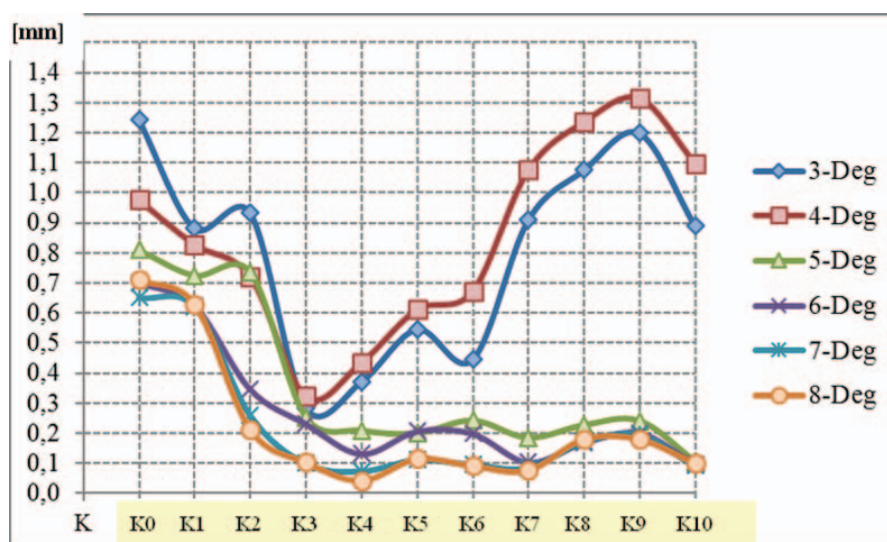


Fig. 5 – Average error fitting deviation diagram.

It is noticeable that the values of the maximum and average deviation of fitted splines of 3rd and 4th degrees diverge. A clear conclusion is that the 3rd and 4th degrees of curves are not appropriate for this patient.

Higher degree splines (6th, 7th, 8th) define a good dental arch of the patient. Table 1 shows that the best result had the 6th degrees spline (10th check-up): maximum deviation was 0.247 mm at the left incisor; average deviation was 0.097 mm. The obtained value of the average deviation was smaller than the accuracy of the used scanner. Figure 6 shows the interpolated spline of 6th degree after the 10th check-up.

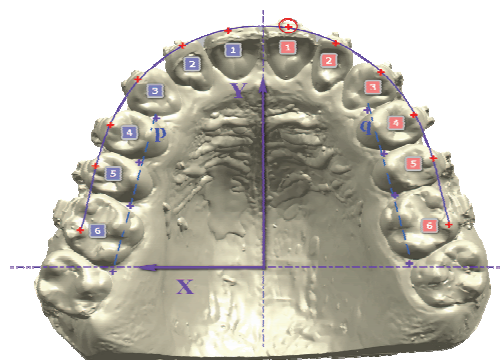


Fig. 6 – Dental arch; 10th control; 6th degrees fitted spline.

Table 1

Values of maximum and average splines fitting errors (Err) in mm

Deg.	Err.	K0	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
3	M	2.642	2.078	1.727	0.820	0.927	1.679	1.188	1.862	2.529	2.175	1.895
	A	1.244	0.882	0.934	0.285	0.372	0.547	0.446	0.910	1.076	1.199	0.891
4	M	2.016	1.879	1.831	0.681	0.935	1.232	1.508	2.205	2.475	2.486	2.186
	A	0.976	0.828	0.721	0.322	0.436	0.612	0.673	1.075	1.236	1.314	1.099
5	M	1.717	1.627	1.337	0.830	0.707	0.397	0.692	0.613	0.804	0.495	0.271
	A	0.811	0.726	0.737	0.253	0.209	0.199	0.243	0.185	0.227	0.242	0.106
6	M	1.615	1.446	0.858	0.566	0.376	0.444	0.404	0.312	0.531	0.435	0.247
	A	0.702	0.623	0.346	0.231	0.132	0.207	0.199	0.104	0.168	0.202	0.097
7	M	1.510	1.308	0.627	0.364	0.173	0.262	0.252	0.226	0.538	0.406	0.219
	A	0.651	0.622	0.260	0.099	0.074	0.112	0.096	0.086	0.172	0.195	0.091
8	M	1.373	1.225	0.483	0.360	0.079	0.253	0.208	0.255	0.544	0.436	0.206
	A	0.711	0.630	0.213	0.103	0.042	0.115	0.091	0.077	0.180	0.180	0.099

Deg. – degrees of fitted splines; “M” and “A” – degrees of interpolated splines. M – maximum fitting error and A – average fitting error. K0, K1, K2, K3, K4, K5, K6, K7, K8, K9, K10 indicate all of the phases of the orthodontics treatment.

## Discussion

The fact is that modern medicine and dentistry cannot be imagined without the involvement of mechanical and electrical engineers. This research is an example of the interdisciplinary engineering modelling and its applications in orthodontics<sup>23</sup>.

There is a large number of studies considering appropriate mathematical equations to define the dental arch. Some of these studies have examined hundreds of patients. The shape of the dental arch depends on gender, age, ethnic background and so on. However, several hundred patients are still too small a sample for the population of a couple of million. It is very difficult and unreliable to try and draw general conclusions because the critics argue that "the sample is too small". Large studies can not generate results regarding an individual. It is necessary for an appropriate government institution to realise this in the next couple of decades.

Due to the previously stated reasons, the authors of this study started from the premise that each individual is a subject unto itself. Personal orthodontics finds the appropriate equation of dental arch for each person separately. For some person 3rd degrees equations will be the most appropriate, for other 6th degrees and so on. The shape of the jaw bone is the most appropriate template for the equation of the dental arch.

It is hard to say which of the displayed curves (in Figure 3) is the most appropriate. Selection of the curve's degree is often very subjective. Is it possible to define an objective mathematical criterion for selection? Is there a mathematical criterion for the orthodontic treatment to be conducted in the desired direction and to the desired objective? The authors developed a method that gives a positive answer to the previous question.

Precise determination of the global coordinate system of the jaw as well as its accurate repeatability<sup>23, 24</sup> is the key to defining the curve that delineates the dental arch. An error in the initial step of determining the coordinate system becomes systematic and cannot be eliminated. If a coordinate system of the jaw is not accurately determined in all successive check-ups, then those check-ups cannot be reliably compared. The obtained results regarding the advancement of orthodontic treatment can be entirely wrong.

Another highly significant aspect is defining and setting of referent geometric entities<sup>2</sup>. They need to be always placed in the same location, in every 3D model generated for

each controls. Random setting, or rule of thumb, does not generate precise results. An error in setting of even a single entity leads to more mistakes it is hard to correct, and even more importantly, difficult to detect.

It is noticeable from the results of the case study that the values of the maximum and average deviation of fitted splines of 3rd and 4th degrees diverge, so they are not appropriate for this patient. The higher degree splines (6th, 7th, 8th) define a good dental arch of the patient. The best fitting of the curve are obtained with the use of 6th degrees spline (10th control – K10): the maximum deviation is 0.219 mm at the left incisor, and the average deviation is 0.091 mm. The obtained value of the average deviation is smaller than the accuracy of the used scanner 23. Such a very small value of the average and maximum deviation of the fitted spline for dental arch curve is also a clear indicator that the treatment is reaching its completion.

## Conclusion

The presented research enable us to define the following conclusions: comparison of dental arch curves is only possible if the jaw coordinate system adjusting was provided with precision and repeatability, and the convergence of the maximum and average deviation of the fitted splines are the criteria for selecting appropriate curve degree. The scanner resolution of at least 0.1 mm and accuracy of 0.2 mm is mathematical convergence criteria.

Described method opens new possibilities of using the digital dental modeling in orthodontic treatment and eliminates a subjective factor as well as make it possible to define the concept of personal orthodontics. Future research into this subject will be conducted by testing this approach in a new clinical study.

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# Natural history of the aortic wall changes in adults with the degenerative tricuspid aortic valve stenosis: the morphometric proofs and implications for echocardiography

Promene aortnog zida kod odraslih osoba sa degenerativnom stenozom trikuspidnog aortnog zaliska: morfometrijski dokazi i implikacije za ehokardiografiju

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## Abstract

**Background/Aim.** So far, no study has been focused exclusively on the tricuspid aortic valve stenosis (TAV) in the aorta without severe dilatation and none has aimed at correlating the high microscopy findings with the echocardiographic parameters. This research was conducted on the postulate that detecting the histopathological changes of different severity in the aortic wall could tailor decision about an aortic surgery. The aim of this study was to grade the histopathological changes in the wall of the nonseverely dilated ascending aorta in patients with the severe, calcific TAV stenosis and to correlate them with the echocardiographic parameters in order to analyze when the ascending aorta should be replaced simultaneously with the aortic valve replacement (AVR). **Methods.** The samples from 37 patients subjected to the AVR and the samples from the control group were analyzed morphologically. The echocardiographic parameters obtained in the TAV stenosis patients were preoperatively correlated with the morphological data, age and gender, diameters of the ventriculo-aortic junction (AA), the sinus Valsalvae (SV) and sinotubular junction (STJ), the largest diameter of the visualized ascending aorta (AscA), the sinus Valsalvae index (SVI) and

AscA/AA index. **Results.** We confirmed morphometrically the exact region of the hemodynamic stress influence with the mathematical distinction in comparison to the controls. In this region, the gradual elastic lamellae disruption was proved by a statistically significant difference through the 3 grades. The elastic skeleton alterations were potentiated with aging and in females. The morphometric parameters of the ascending aorta wall statistically significantly correlated with the echocardiographic parameters: AA, SV, AscA and SVI. The echocardiographic parameters tended to be higher in the most severe grade 3, in the patients younger than 65 years of age. The AscA of more than 4.5 cm was associated with the irreversible morphological defects in these patients. **Conclusion.** The hemodynamic stress induced by the TAV stenosis leads to the ascending aorta elastic lamellae disruption that could be histopathologically graded and correlated with the echocardiographic parameters of the ascending aorta providing a potential tool for decision-making process in cases when the ascending aorta replacement is considered simultaneously with the AVR.

## Key words:

aorta; aging; aortic valve stenosis; echocardiography; heart valve prosthesis; histological techniques; tissues.

## Apstrakt

**Uvod/Cilj.** Istraživanja usmerena isključivo na stenozu trikuspidalnog aortnog zaliska aorte bez teške dilatacije nisu rađena, kao što nijedna studija nije imala za cilj korelaciju

nalaza svetlosne mikroskopije sa ehokardiografskim parametrima. Ovo istraživanje je sprovedeno na osnovu pretpostavke da nalaženje patohistoloških promena u zidu aorte, različitog stepena težine, može uticati na kreiranje strategije operisanja aorte. Cilj rada je bio da se kod bolesnika sa

teškom degenerativnom stenozom trikuspidnog aortnog zaliska stepenuju patohistološke promene zida ascendentne aorte, koja nije bila značajno dilatirana i korelišu sa ehokardiografskim parametrima, u cilju procene pogodnog vremena simultane zamene ascendentne aorte i aortnog zaliska. **Metode.** Morfološkom analizom je bilo obuhvaćeno 37 uzoraka, uzetih od bolesnika kod kojih je vršena operacija zamene aortne valvule i od kontrolne grupe ispitanika. Ehokardiografski parametri [dijametri aorte na nivou ventrikulo-aortnog spoja (AA), sinusa Valsalve (SV) i sinotubularnog spoja (ST)], najveći dijametar ascendentne aorte (AscA), indeks sinusa Valsalve (SVI) i AscA/AA indeks], izmereni kod bolesnika sa stenozom aortne valvule, bili su korelisani sa morfološkim nalazom, starošću i polom ispitanika. **Rezultati.** Morfometrijskom analizom potvrđeno je da su reprezentativni uzorci prikupljeni iz regiona najvećeg hemodinamskog stresa. Potvrđeno je progresivno oštećenje elastičnih lamela u tri različita gradusa. Potvrđeno je veće oštećenje elastičnih lamela kod starijih osoba i kod žena. Morfome-

trijski parametri zida ascendentne aorte korelisali su značajno sa ehokardiografskim parametrima: AA, SV, AscA i SVI. Vrednosti izmerenih ehokardiografskih parametara bile su veće kod bolesnika sa najtežim gradusom morfometrijskih promena (gradus 3), mlađih od 65 godina. Vrednost AscA veća od 4,5 cm bila je udružena sa ireverzibilnim morfološkim promenama. **Zaključak.** Hemodinamski stres uzrokovao stenozom aortnog zaliska dovodi do oštećenja elastičnih lamela zida aorte koja se mogu gradirati patohistološki i korelisati sa ehokardiografskim parametrima ascendentne aorte što obezbeđuje dodatni kriterijum za odlučivanje kod onih bolesnika kod kojih se razmatra zamena aortnog zaliska istovremeno sa zamenom ascendentne aorte.

#### Ključne reči:

**aorta; starenje; zalistak, aortni, stenoza; ehokardiografija; zalisci srca, veštački; histološke tehnike; tkiva.**

## Introduction

The influence of a stenotic jet and the severity of the tricuspid aortic valve (TAV) stenosis to the aortic wall changes and to the aortic root and ascending aorta parameters have not been explicitly and unambiguously determined<sup>1</sup>. Only few studies investigated the histopathological defects of the elastic skeleton in patients with the aortic valve dysfunction with a limited number of the elastic skeleton parameters<sup>2-5</sup>. However, none was focused exclusively on the TAV stenosis in the aortas without severe dilatation, and none aimed at correlating the light microscopy findings with the echocardiographic parameters.

In the present study, we investigated the spectrum of structural changes in the ascending aorta walls of the patients with the severe degenerative, calcific aortic stenosis of the TAV. Recognition of the evolution of the aortic wall changes due to the aortic stenosis is essential for tailoring the surgical guidelines for the degenerative aortic stenosis treatment. The main goal of our study was to evaluate whether and when the replacement of the ascending aorta is warranted simultaneously with the aortic valve replacement (AVR).

A design of the study was led by several questions. First, whether there were some changes in the basic structure of the ascending thoracic aorta due to the severe stenosis of the TAV? Second, whether there was a gradual progression of those changes, caused by the hemodynamic disturbances in the setting of the aortic stenosis? Third, whether we could apply a grading system for the aortic wall changes? And more importantly, whether we could find the irreversible changes in the ascending aorta wall in the patients with the severe aortic stenosis, but with no dilated aorta? Finally, whether we could correlate a histological grade with the echocardiographic parameters in order to obtain a reliable insight into the aortic wall structure by means of the noninvasive diagnostics?

Apart from the hemodynamic derangement that the aortic stenosis causes, aging has a potential for the degenerative

changes of the ascending aorta. The next question was whether we could distinguish the aging-induced changes from the aortic stenosis-induced changes. Eventually, we analyzed if there were some gender-related differences in the remodeling process.

We focused on the severe TAV stenosis and its influence on the ascending aorta wall. The chosen method to assess the aortic wall changes in this study was the analysis of the elastic skeleton parameters. We included a wide range of parameters comprising the internal elastic membrane parameters, elastic lamellae parameters and interlamellar fibers parameters.

## Methods

### Overall patients data

The wall segments of the ascending aorta of 30 patients who were undergoing the AVR, because of the severe, symptomatic TAV stenosis, were analyzed at the Dedinje Cardiovascular Institute, Belgrade, Serbia from August 2005 to April 2009. There were 14 (46.7%) male and 16 (53.3%) female patients. The mean age of the patients was  $64.1 \pm 7.8$  years. The diameter of the ascending aorta was  $< 5$  cm in all patients, with the mean value of  $3.49 \pm 0.63$  cm. The minimal diameter was 2.6 cm and the maximal diameter 4.7 cm. We aimed at investigating the ascending aortas that were  $< 5.5$  cm in diameter being the cut-off value for the indication for the aortic root surgery in the patients with the TAV stenosis<sup>1</sup>. Our classification of the nonseverely dilated ascending aorta, stressed out that the patients in our group had much smaller diameter than the patients indicated for a surgery according to the existing guidelines.

The exclusion criteria from this study group were the patients with the moderate or severe aortic regurgitation, aortic stenosis and acute or chronic aortic dissection, the patients who previously had a cardiac operation and the ones who had the aortic stenosis combined with a connective tissue disorder, bicuspid or congenitally malformed the aortic valve.

The aortic wall samples in the control group were obtained during the autopsies at the Institute of Pathology, Faculty of Medicine, University of Belgrade from the patients who died of noncardiac and nonvascular causes from April 2009 to May 2009. In the control group, there were 7 patients, 3 (42.9%) males and 4 (57.1%) females. The mean age of the patients in the control group was  $68.9 \pm 14.2$  years. Exclusion criteria were the same as for the study group.

The study was approved by the Ethics Committee of the Dedinje Cardiovascular Institute. Informed consent was obtained from all patients.

#### *Intraoperative aortic wall sampling*

The diagnosis of a severe TAV stenosis was established by preoperative echocardiography<sup>5</sup>. Transverse aortotomy was made approximately 1 cm above the take-off of the right coronary artery, slightly above the level of the sinotubular junction. The aortic wall specimens were taken from the distal lip of the incision at the convexity of the ascending aorta, 2 to 4 cm above the level of the aortic valve annulus<sup>6</sup>. The samples of the aortic wall with the minimal dimensions of 1 mm  $\times$  9 mm and maximal dimensions of 3 mm  $\times$  12 mm were excised, immediately fixed in 4% neutral buffered formaldehyde by the immersion procedure, and subsequently processed for the morphological and morphometric analysis.

During the autopsies, the matched control samples were obtained from the same region. For the control group, we chose the samples with intimal hyperplasia or the atherosclerotic lesion types I to III in the selected region.

#### *The echocardiographic parameters*

The echocardiographic parameters of the aortic root and the ascending aorta were determined preoperatively from the parasternal longitudinal section according to the standard 2D procedure. The diameters at the level of ventriculo-aortic junction (AA), sinus Valsalvae (SV), sinotubular junction (STJ) and the largest diameter of the visualized ascending aorta (AscA) were measured.

The index of the sinus Valsalvae (SVI) was calculated as the ratio between measured and predicted diameter (pSV) at the level of the sinus of Valsalva. The predicted diameter at the level of the sinus Valsalvae (pSV) was calculated according to the regression formula  $pSV \text{ (cm)} = 1.92 + 0.74 \times BSA \text{ (m}^2\text{)}$ , where the BSA stands for body surface area<sup>5</sup>.

#### *Preparation of the arterial samples for the analysis*

The tissue was prepared for the morphological and morphometric analysis according to the procedure described in the previously published studies of our group<sup>6,7</sup>. Out of 30 serial sections per patient, 3 sections were chosen for the morphometric analysis with respect to the following several rules: the oblique sections were excluded from the analysis as well as the sections with major technical flaws. Also, a minimal distance between chosen sections had to be at least 100  $\mu\text{m}$ .

The sections were stained applying some selective techniques for the elastic fibers: the Weigert van Gieson technique with resorcin fuchsin, the Verhoeff van Gieson method, or the Pincus' staining with acid orcein.

All sections were graded according to the principle described by Schlatmann and Becker<sup>8</sup> for gradation of the aortic wall changes during aging and Niwa et al.<sup>9</sup> for gradation of the congenital aortic stenosis. In both analyses we confirmed the statistically significant differences among the grades. However, we decided to proceed with the Schlatmann and Becker<sup>8</sup> gradation system since the criteria for grading were more precise, hence the reproducibility of the results was also higher with this system.

The grades were established according to the most severe changes on the magnification  $\times 200$  of the Olympus BX41 microscope.

Grade 1 is present if there are fewer than 5 foci of the elastic lamellae fragmentation in 1 microscopic field. Focus of the elastic lamellae fragmentation comprises 2 to 4 neighboring elastic lamellae. The orientation of the smooth muscle cells is preserved (Figures 1 A–B).

Grade 2 means that 5 to 10 foci of the elastic lamellae fragmentation in 1 microscopic field and foci are confluent or scattered throughout the media of the aorta. Each focus comprises 2 to 4 neighboring elastic lamellae. The orientation of the smooth muscle cells is preserved (Figures 1 C–D).

Grade 3 is present if there are foci with the elastic fragmentation in 5 or more neighboring elastic lamellae, irrespective of the number of foci per microscopic field. The smooth muscle cells of the media show alterations in orientation (Figures 1 E–F).

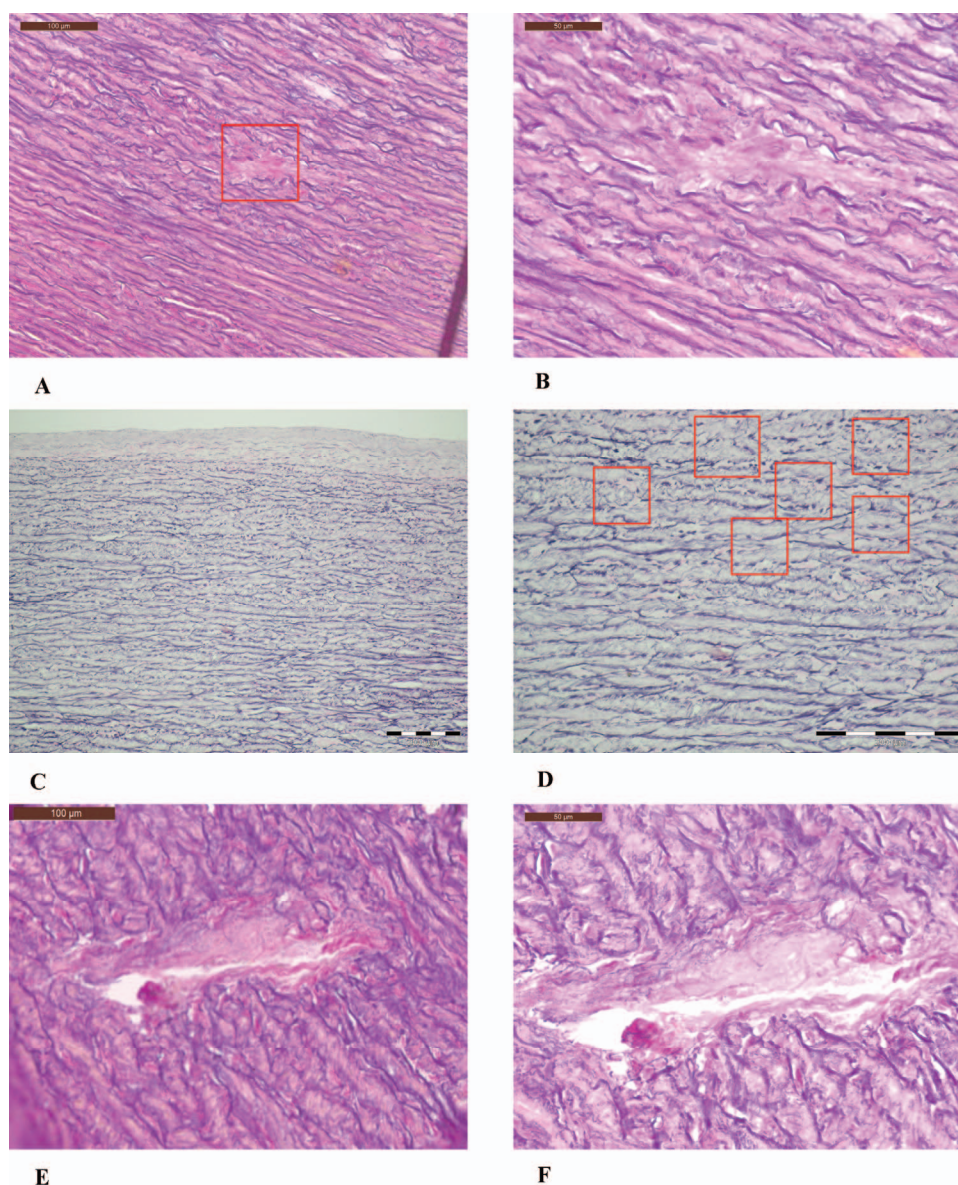
A pathologist, who performed the analysis, was blind for the patient's data. The slides were reexamined twice to obtain the final data as advised in similar studies<sup>10</sup>.

#### *Morphometric analysis*

Two sets of the morphometric parameters were measured.

The following morphometric parameters were measured to analyze the lesions induced by the hemodynamic influence of the severe TAV stenosis and to confirm the remodeling pattern of the lesions: the tunica intima thickness, the maximal tunica intima thickness, the tunica media thickness, the tunica adventitia thickness and the aortic wall thickness.

The following parameters of the elastic skeleton were measured to confirm the morphometric differences among the grades: the maximal thickness of the internal elastic lamina (IEL), the number of the IEL fenestrations per 50  $\mu\text{m}$ , the size of the IEL fenestrations, the number of the elastic lamellae per 50  $\mu\text{m}$ , the maximal thickness of the elastic lamellae, the number of the elastic lamellae fenestrations per 50  $\mu\text{m}$ , the size of the elastic lamellae fenestrations, the number of the interlamellar elastic fibers per 50  $\mu\text{m}$  of the length of the interlamellar unit, the maximal thickness of interlamellar fibers, the number of fenestrations per interlamellar fiber and the size of fenestrations of interlamellar fibers



**Fig. 1 – The ascending aorta in the patients with the degenerative tricuspid aortic valve stenosis – A) and E) Weigert van Gieson staining,  $\times 200$ , bar = 100  $\mu\text{m}$ ; B) and F) Weigert van Gieson staining,  $\times 400$ , bar = 50  $\mu\text{m}$ ; C) PTAH staining,  $\times 100$ , bar = 200  $\mu\text{m}$ ; D) PTAH staining,  $\times 200$ , bar = 200  $\mu\text{m}$ ; A)–B) grade 1; one focus of the elastic lamellae fragmentation in 1 microscopic field of the Olympus BX41 microscope,  $\times 200$ ; a focus of the elastic lamellae fragmentation comprises 2 to 4 neighboring elastic lamellae; orientation of the smooth muscles is preserved; C)–D) grade 2; 6 foci of the elastic lamellae fragmentation in 1 microscopic field of the Olympus BX41 microscope,  $\times 200$ ; the confluent or scattered foci throughout the media of the aorta; orientation of the smooth muscle cells is preserved; E)–F) the presence of foci with the elastic fragmentation in 5 or more neighboring elastic lamellae with a disruption of the smooth muscle cells.**

The optical microscope Olympus BX41 with the Olympus C5060-ADU wide zoom camera and the Olympus DP-software Analyzer program was used for the morphometric analysis.

The thickness of the tunica intima, the tunica media, the tunica adventitia and that of the wall were measured as described in our previous publications<sup>11</sup>. The intima-media ratio was calculated for the purpose of the correlation with the echocardiographic parameters.

The systematic field sampling method was used<sup>3, 7</sup>. Agrids with thirty-five 50  $\mu\text{m} \times 50 \mu\text{m}$  fields was embedded into each microscopic field. For each patient, the mor-

phometric parameters of the elastic lamellae and interlamellar fibers were measured on 10 microscopic fields, that is, three hundred and fifty 50  $\mu\text{m} \times 50 \mu\text{m}$  fields per section. The thickness of the elastic lamellae was measured in an axis perpendicular to the elastic lamellae. Each lamella was measured in 3 to 6 different positions and only the maximal thickness was used for further statistical processing. We choose only ideal cross-sections with the perfect contours of the elastic lamellae. The fields with the membrane reduplication were excluded from the analysis. Afterwards, the number of fenestrations per 50  $\mu\text{m}$  and their size were meas-

ured. The size of fenestrations was measured as the shortest distance between the two fragments of lamellae. Major defects of the grade 3 were not considered as fenestrations, neither were they measured. These gaps were used for the gradation purposes only (Figures 1E–F), while defined fenestrations in the preserved lamellae were included into the morphometric analysis.

After that, the maximal thickness of the interlamellar fibers was measured as well as the number of fenestrations and their size.

The elastic lamellae and interlamellar fibers parameters were measured at the magnification  $\times 400$  of the Olympus BX41 microscope.

### *Statistical analysis*

Descriptive statistics included the mean values and the median, the standard deviation (SD), the standard error (SE) and a 95% confidence interval (95% CI).

The tests were performed with the SPSS version 10.0 for Windows. The following tests were used where appropriate: one-sample Kolmogorov-Smirnov test,  $\chi^2$  test, Mood's median test, ANOVA, Mann-Whitney test, Kruskal-Wallis test, post-hoc multiple comparisons LSD test and Bonferroni multiple comparison test, Pearson correlation and Spearman's Rho. The value of  $p < 0.05$  was considered statistically significant.

The data are presented as the means  $\pm$  SD or the means  $\pm$  SE.

The data distribution pattern was analyzed with the one-sample Kolmogorov-Smirnov test. The values of the tunica media, tunica adventitia and wall thickness were distributed according to the normal distribution and analyzed with the parametric tests. The values of the thickness of the tunica intima, maximal thickness of the tunica intima and the intima-to-media ratio were not distributed according to the normal distribution. The values of these parameters were transformed and the  $\ln$  values of the tunica intima thickness, maximal tunica intima thickness and the intima-to-media were analyzed for the normality of their distribution (Table 1). The transformed values of the tunica intima thickness and the maximal tunica intima thickness had a normal distribution, they were analyzed by the parametric tests and the presented results apply to these values. The intima-to-media ratio values had no normal distribution and they were analyzed by the non-parametric tests.

The data distribution pattern was analyzed by the One-Sample Kolmogorov-Smirnov test. The values of the elastic lamellae parameters did not conform the normal distribution, hence the non-parametric tests were used in the analysis.

When the data were grouped into the age-related groups ( $< 65$  years and  $\geq 65$  years), the normal distribution was confirmed for the number of the elastic lamellae and for the thickness of the elastic lamellae and interlamellar fibers. These data were analyzed using the ANOVA test.

When the data were grouped according to the gender, the normal distribution was confirmed for the number of the

elastic lamellae and for the thickness of elastic lamellae. These data were analyzed using the ANOVA test.

A normal distribution of the values of the echocardiographic parameters was confirmed by the one-sample Kolmogorov-Smirnov test, hence they were analyzed with the parametric tests.

## **Results**

### *Remodeling in the degenerative aortic stenosis is associated with a significant thinning of the ascending aorta wall*

#### **Remodeling pattern**

A significant thinning of the ascending aorta walls and all of its tunics is characteristic of the remodeling pattern in the degenerative aortic stenosis as confirmed by the morphometric analysis (Figures 2 and 3A and Table 1). The thickness of all parameters, except the thickness of the tunica adventitia were statistically significantly smaller than in the age and atherosclerosis matched controls as confirmed by the ANOVA or Mann-Whitney tests (Figure 2 and Table 1).

These results confirmed that the jet produces changes of the aortic wall, distinctive both morphologically and mathematically. Consequently, it was used to prove that the samples were taken from the representative area of the aortic wall, the finding truly important since the geometry of the stenotic aortic orifice is complex, its influence on the jet direction is unpredictable and the aortic circumference is not equally affected in all of its parts. When the post-hoc Bonferroni multiple comparison test, or the Kruskal-Wallis test were applied, the differences between the controls and the aortic stenosis group were confirmed (Figure 2).

### *The morphometric parameters follow the grading pattern in the aortic stenosis*

The morphometric parameters were significantly different among the grades of the aortic stenosis group as confirmed by the ANOVA, or Mann-Whitney test (Figure 2 and Table 1). The gradual increase in the thickness of the aortic wall and its layers from the grade 1 to grade 3 was observed, consistent with the gradual decay of the elastic skeleton which was proved in the further analysis. Nevertheless, the post-hoc Bonferroni multiple comparison test did not confirm differences among the grades for all parameters (Figure 2), so the linear correlation tests were used for further analysis.

Additional testing with the Pearson's correlation coefficient or Spearman's Rho confirmed trending obtained with the ANOVA, or Mann-Whitney tests. The following parameters correlated positively and were statistically significant to the grades: the thickness of the tunica intima ( $p < 0.001$ ), the maximal thickness of the tunica intima ( $p < 0.001$ ), the wall thickness ( $p = 0.023$ ) and the tunica adventitia thickness ( $p = 0.029$ ) as well as the intima-media ratio ( $p < 0.001$ ). The tunica media thickness did not correlate significantly with the grades ( $p = 0.382$ ).

Table 1

## Values of the tunica media, tunica adventitia and the aortic wall thickness of the aortic stenosis and control groups

Parameter	Means $\pm$ SD	95% CI	Min–Max
The thickness of the tunica intima ( $\mu\text{m}$ )			
control group	226.51 $\pm$ 127.18	108.88–344.13	55.31–479.05
aortic stenosis group	33.02 $\pm$ 41.06	16.08–49.97	3.89–169.18
ANOVA = 17.451, $p < 0.001^{**  }$			
GR 1	14.09 $\pm$ 9.97	6.96–21.22	3.89–35.31
GR 2	20.97 $\pm$ 9.00	14.53–27.40	9.09–35.81
GR 3	95.01 $\pm$ 60.39	20.03–169.99	37.49–169.18
The maximal thickness of the tunica intima ( $\mu\text{m}$ )			
control group	371.14 $\pm$ 191.54	193.99–548.28	82.35–618.86
aortic stenosis group	74.19 $\pm$ 90.60	36.80–111.59	8.77–329.43
ANOVA = 11.686, $p < 0.001^{**  }$			
GR 1	30.84 $\pm$ 29.82	9.50–52.17	8.77–103.53
GR 2	47.58 $\pm$ 17.27	35.22–59.93	29.67–75.43
GR 3	214.14 $\pm$ 124.98	58.97–369.32	68.40–329.43
The thickness of the tunica media ( $\mu\text{m}$ )			
control group	1,380.37 $\pm$ 201.34	1,194.17–1,566.58	1,069.13–1,628.03
aortic stenosis group	1,041.46 $\pm$ 199.08	959.28–1,123.64	593.04–1,382.86
ANOVA = 5.757, $p = 0.0382^{**}$			
GR 1	975.45 $\pm$ 241.08	803.00–1,147.91	593.04–1,245.25
GR 2	1,106.76 $\pm$ 148.77	1,000.33–1,213.18	939.07–1,382.86
GR 3	1,042.87 $\pm$ 188.88	808.34–1,277.40	728.88–1,211.31
The thickness of the wall ( $\mu\text{m}$ )			
control group	2,028.21 $\pm$ 307.63	1,743.70–2,312.72	1,658.59–2,534.36
aortic stenosis group	1,279.96 $\pm$ 264.50	1,168.28–1,391.65	666.1–1,862.40
ANOVA = 13.697, $p < 0.023^{**}$			
GR 1	1,122.77 $\pm$ 325.31	872.72–1,372.82	666.51–1,670.16
GR 2	1,349.11 $\pm$ 117.59	1,264.99–1,433.23	1,208.75–1,529.03
GR 3	1,424.63 $\pm$ 256.50	1,106.14–1,743.11	1,193.96–1,862.40
The thickness of the tunica adventitia ( $\mu\text{m}$ )			
control group	422.55 $\pm$ 187.75	70.96–248.91	266.39–753.13
aortic stenosis group	216.28 $\pm$ 163.69	147.16–285.40	61.25–812.84
ANOVA = 2.822, $p = 0.029$			
GR 1	130.94 $\pm$ 103.64	51.27–210.60	61.25–390.84
GR 2	242.02 $\pm$ 64.85	195.63–288.41	180.09–349.74
GR 3	318.43 $\pm$ 303.38	58.27–695.13	61.98–812.84
The intima to media ratio			
control group	0.173 $\pm$ 0.104	0.077–0.269	0.040–0.370
aortic stenosis group	0.032 $\pm$ 0.036	0.018–0.047	0.010–0.150
Mann-Whitney test = 11.000, $p < 0.001^{**\ddagger}$			
GR 1	0.021 $\pm$ 0.012	0.014–0.028	0.010–0.040
GR 2	0.020 $\pm$ 0.007	0.011–0.029	0.010–0.030
GR 3	0.070 $\pm$ 0.059	0.034–0.092	0.010–0.037

GR – grade; SD – standard deviation; CI – confidence interval; Min – minimum; Max – maximum.

||ANOVA test is applied to ln (thickness of the tunica intima) or ln (maximal thickness of the tunica intima);

\*\*statistically significant difference;  $\ddagger$  non-parametric tests applied.

ured. The size of fenestrations was measured as the shortest distance between the two fragments of lamellae. Major defects of the grade 3 were not considered as fenestrations, neither were they measured. These gaps were used for the gradation purposes only (Figures 1E–F), while defined fenestrations in the preserved lamellae were included into the morphometric analysis.

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A normal distribution of the values of the echocardiographic parameters was confirmed by the one-sample Kolmogorov-Smirnov test, hence they were analyzed with the parametric tests.

## **Results**

### *Remodeling in the degenerative aortic stenosis is associated with a significant thinning of the ascending aorta wall*

#### **Remodeling pattern**

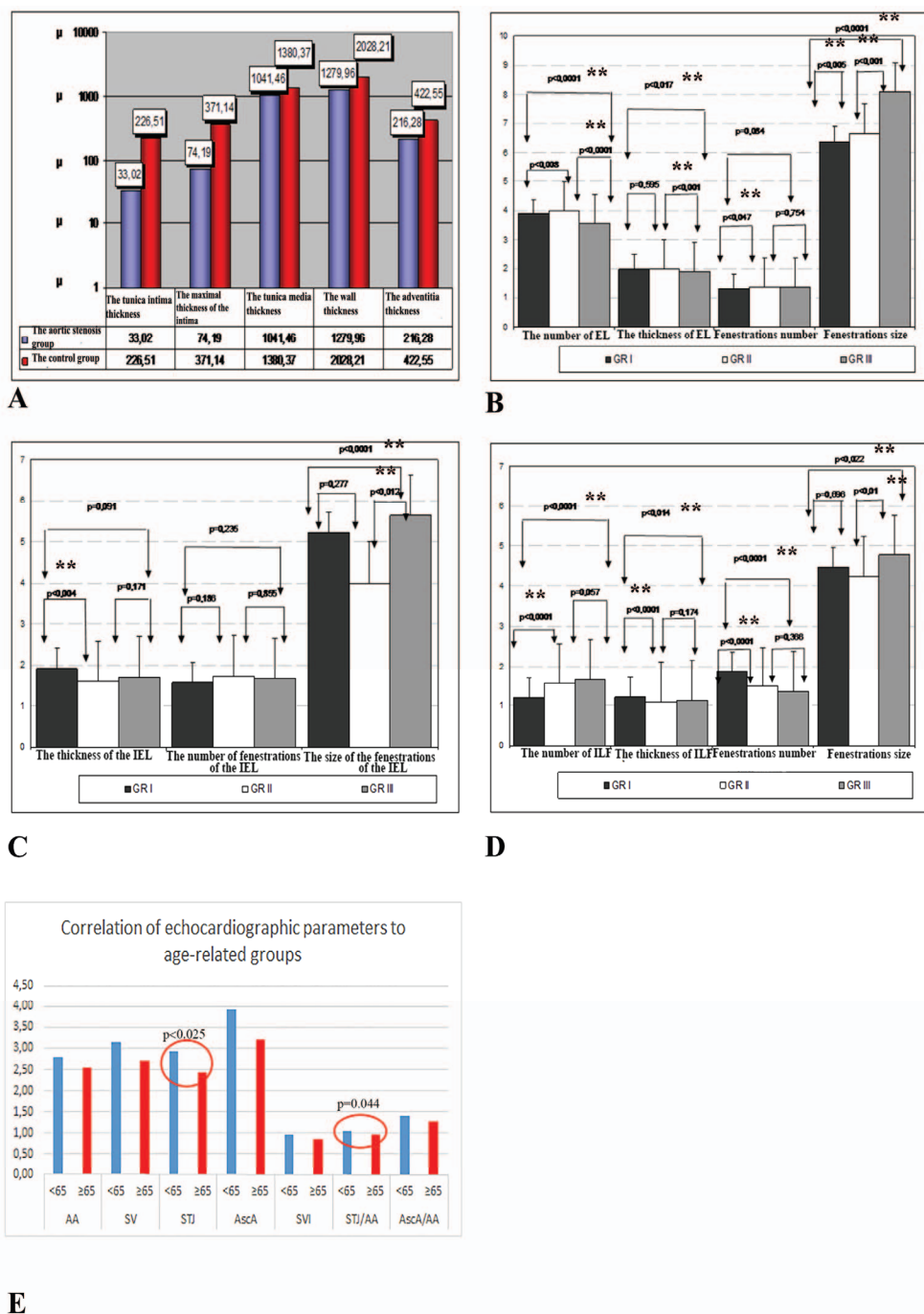
A significant thinning of the ascending aorta walls and all of its tunics is characteristic of the remodeling pattern in the degenerative aortic stenosis as confirmed by the morphometric analysis (Figures 2 and 3A and Table 1). The thickness of all parameters, except the thickness of the tunica adventitia were statistically significantly smaller than in the age and atherosclerosis matched controls as confirmed by the ANOVA or Mann-Whitney tests (Figure 2 and Table 1).

These results confirmed that the jet produces changes of the aortic wall, distinctive both morphologically and mathematically. Consequently, it was used to prove that the samples were taken from the representative area of the aortic wall, the finding truly important since the geometry of the stenotic aortic orifice is complex, its influence on the jet direction is unpredictable and the aortic circumference is not equally affected in all of its parts. When the post-hoc Bonferroni multiple comparison test, or the Kruskal-Wallis test were applied, the differences between the controls and the aortic stenosis group were confirmed (Figure 2).

### *The morphometric parameters follow the grading pattern in the aortic stenosis*

The morphometric parameters were significantly different among the grades of the aortic stenosis group as confirmed by the ANOVA, or Mann-Whitney test (Figure 2 and Table 1). The gradual increase in the thickness of the aortic wall and its layers from the grade 1 to grade 3 was observed, consistent with the gradual decay of the elastic skeleton which was proved in the further analysis. Nevertheless, the post-hoc Bonferroni multiple comparison test did not confirm differences among the grades for all parameters (Figure 2), so the linear correlation tests were used for further analysis.

Additional testing with the Pearson's correlation coefficient or Spearman's Rho confirmed trending obtained with the ANOVA, or Mann-Whitney tests. The following parameters correlated positively and were statistically significant to the grades: the thickness of the tunica intima ( $p < 0.001$ ), the maximal thickness of the tunica intima ( $p < 0.001$ ), the wall thickness ( $p = 0.023$ ) and the tunica adventitia thickness ( $p = 0.029$ ) as well as the intima-media ratio ( $p < 0.001$ ). The tunica media thickness did not correlate significantly with the grades ( $p = 0.382$ ).



**Fig. 3 – A)** Comparison between the aortic stenosis group and the control group according to the thickness of the aortic wall and its layers – notice a significant thinning of the wall and all tunics; **B)** comparison of the different grades (GR): the morphometric parameters of the elastic lamellae – a statistically significant decrease in the thickness and the number of the elastic lamellae between the GR1 and GR3 and a statistically significant increase in the size of the GRs (GR1 vs. GR2, GR1 vs. GR2, GR2 vs. GR3) and the number of fenestrations of the elastic lamellae among (GR1 vs. GR2); **C)** comparison of different GRs: the morphometric parameters of the IEL – a statistically significant decrease in the thickness of the IEL (GR 1 vs. GR2) and a statistically significant increase in the size of fenestrations of the IEL (GR1 vs. GR 3; GR2 vs. GR3); **D)** comparison of different GRs: the morphometric parameters of the interlamellar fibers – a statistically significant decrease in the interlamellar fibers thickness (GR1 vs. GR2; GR1 vs. GR3) and the number of interlamellar fibers fenestrations (GR1 vs. GR2; GR1 vs. GR3) and a statistically significant increase in the number of the interlamellar fibers (GR1 vs. GR2; GR1 vs. GR3) and the size of interlamellar fibers fenestrations (GR1 vs. GR3; GR2 vs. GR3); **E)** the diameter at the sinotubular junction (STJ) and the STJ/AA index correlated statistically significantly with the age-related groups.

AA – diameters at the level of the ventriculo-aortic junction, SV – diameters of the sinus of Valsalva, STJ – diameters at the level of the sinotubular junction, AscA – the largest diameter of the visualized ascending aorta, SVI – the sinus of Valsalva index.

*The elastic lamellae changes follow the progression and natural history of the aortic stenosis*

The analysis of the elastic lamellae aimed at proving that there was a significant difference in the elastic skeleton parameters among the different grades and consequently grades following the progression of the degenerative changes of the aortic wall caused by the aortic stenosis. Furthermore, morphometry of the elastic lamellae aimed at proving that the established grades were accurate and specific enough to detect the real histopathological changes caused by the aortic stenosis and not by aging. Finally, morphometry of the elastic lamellae aimed at identifying the irreversible changes. It was important to confirm the difference between each grade and the control group in that respect.

The remodeling pattern – the elastic lamellae changes follow the grading pattern

Firstly, the elastic lamellae parameters and their differences in the aortic stenosis group and the control group were compared. It was proved by the Mood's median test and non-parametric Z test as the post-hoc test that the remodeling of the elastic lamellae in the aortic stenosis was associated with augmentation of the elastic lamellae as compared to the control group (Table 2). The elastic lamellae were significantly thicker and more numerous, while the size of their fenestrations was reduced. The aortic stenosis obviously induced up-

grading of the elastic lamellae, in comparison to the control group with a statistical significance ( $p < 0.001$ ).

Secondly, the elastic lamellae changes among different grades of the aortic stenosis group were studied. Among the different grades in the aortic stenosis group, a significant degradation of the elastic lamellae was confirmed by applying the Mood's median test ( $p < 0.001$ ) and the non-parametric Z test as the post-hoc test. The number of the elastic lamellae and their thickness decreased significantly while the number of elastic lamellae fenestrations and their size increased significantly (Table 2 and Figure 3B).

Later regularity was also observed when the parameters of the IEL were analyzed (Figure 3C).

Remodeling pattern – evolution of the interlamellar fibers changes follows the grading pattern

The reorganization of the interlamellar fibers was also detected in comparison to the aortic stenosis group and the control group. The number of the interlamellar fibers and the size of interlamellar fibers fenestrations was significantly higher in the control group when compared to the aortic stenosis group (Table 3). The interlamellar fibers thickness was significantly lower in the control group in comparison to the aortic stenosis group while the number of interlamellar fibers fenestrations did not differ between the control group and the aortic stenosis group (Table 3).

**Table 2**

**Morphometric parameters of the elastic lamellae – comparison between the control group and the grades of the aortic stenosis group**

Parameters of the elastic lamellae	N	Mean $\pm$ SD	Min–Max	$p$
The number				
control	504	3.33 $\pm$ 0.96	3.25–3.42	< 0.001**
GR 1	1,311	4.46 $\pm$ 1.56	4.37–4.54	
GR 2	1,831	3.54 $\pm$ 1.31	3.48–3.59	
GR 3	453	3.27 $\pm$ 1.18	3.16–3.37	
The thickness ( $\mu$ m)				
control	305	1.56 $\pm$ 0.52	1.50–1.62	< 0.001**
GR 1	973	2.20 $\pm$ 0.65	2.16–2.24	
GR 2	1,400	1.84 $\pm$ 0.61	1.81–1.87	
GR 3	306	1.85 $\pm$ 0.62	1.79–1.92	
The number of fenestrations				
control	432	1.20 $\pm$ 0.79	1.13–1.28	< 0.001**
GR 1	1,195	1.32 $\pm$ 0.87	1.27–1.37	
GR 2	1,392	1.35 $\pm$ 0.94	1.31–1.40	
GR 3	326	1.46 $\pm$ 0.90	1.37–1.55	
The size of fenestrations ( $\mu$ m)				
control	526	10.59 $\pm$ 6.83	10.01–11.18	< 0.001**
GR 1	1,352	6.33 $\pm$ 4.22	6.11–6.56	
GR 2	1,699	7.17 $\pm$ 4.73	6.98–7.37	
GR 3	433	7.68 $\pm$ 5.28	7.23–8.13	

**A statistically significant increase in the thickness of elastic lamellae between the control group and the aortic stenosis group; there is a statistically significant difference among different grades in the elastic lamellae thickness; the size of fenestrations decreased significantly between the control group and the aortic stenosis group; the size of elastic lamellae fenestrations increased significantly among the grades; the number of the elastic lamellae increased significantly between the aortic stenosis group and the control group; there is a significant decrease in the elastic lamellae number among the grades; the number of the elastic lamellae fenestrations increased significantly between the control group and the grade 3.**

**GR – grade; SD – standard deviation; \*\* $p$  – statistically significant difference.**

**Table 3**

**Morphometric parameters of the interlamellar fibers – comparison between the control group and the grades of the aortic stenosis group**

Parameter of interlamellar fibers	N	Mean $\pm$ SD	95% CI	<i>p</i>
The number				
control	252	1.58 $\pm$ 0.68	1.50–1.67	< 0.004**
GR 1	479	1.20 $\pm$ 0.84	1.14–1.27	
GR 2	725	1.56 $\pm$ 0.77	1.49–1.64	
GR 3	236	1.66 $\pm$ 0.82	1.60–1.73	
The thickness ( $\mu$ m)				
control	361	0.85 $\pm$ 0.33	0.82–0.89	< 0.001**
GR 1	501	1.21 $\pm$ 0.53	1.17–1.25	
GR 2	790	1.09 $\pm$ 0.40	1.06–1.12	
GR 3	189	1.13 $\pm$ 0.46	1.10–1.17	
The number fenestrations				
control	61	1.45 $\pm$ 0.78	1.25–1.65	= 0.122
GR 1	235	1.85 $\pm$ 1.05	1.73–1.97	
GR 2	309	1.48 $\pm$ 0.86	1.36–1.59	
GR 3	77	1.37 $\pm$ 0.68	1.28–1.45	
The size of fenestration				
control	88	5.29 $\pm$ 2.76	4.70–5.87	< 0.002**
GR 1	341	4.46 $\pm$ 2.42	4.25–4.68	
GR 2	451	4.23 $\pm$ 1.93	4.00–4.45	
GR 3	128	4.77 $\pm$ 2.52	4.49–5.05	

For abbreviations see under Tables 1 and 2.

**A statistically significant increase in the thickness of interlamellar fibers between the control group and the aortic stenosis group; the interlamellar fibers thickness decreased statistically significantly among grades GR1 and GR3; the size of fenestrations decreased significantly between the control group and the aortic stenosis group; the size of interlamellar fibers fenestrations increased significantly between grades GR1 and GR3; the number of the interlamellar fibers is proved to have no significance in the context of the multiple comparison.**

In the aortic stenosis group, the number of the interlamellar fibers and their thickness increased significantly among grades, as did the number of elastic lamellae fenestrations and their size. The number of interlamellar fibers fenestrations decreased in the subsequent grades (Table 3 and Figure 3D).

#### Influence of aging

Out of 30 patients in the aortic stenosis group, 13 (43.3%) were younger than 65 years of age while 17 (56.7%) were older than 65 years of age. There was no statistically significant difference among the number of the patients in the group younger than 65 years of age, and group older than 65 years of age ( $\chi^2 = 0.533$ ,  $p = 0.465$ ). Further analysis showed that the ageing affects all parameters of the elastic skeleton with the statistical significance, except the number of fenestrations of the elastic lamellae as proved by the ANOVA or Z test (Table 4).

The changes were more severe in the inner media, and there seemed to be a progression of the changes from the inwards to the outwards of the aortic media (Figures 1B and 1C).

The next question was whether we could distinguish effects of aging and the aortic stenosis, or the described grades were merely misapprehended changes induced by ageing. The ANOVA or Kruskal-Wallis tests were applied to test the differences between the control group and the aortic stenosis

group of patients younger than 65 years of age and in the group of patients older than 65 years of age. It was confirmed that a statistical significance persisted (Table 5). Since the established differences were the effects of the aortic stenosis, they were potentiated with ageing, but they were not entirely dependable on ageing. The number of the elastic lamellae fenestrations changed intensively with ageing. The size of the elastic lamellae fenestrations had even greater alterations in the patients younger than 65 years of age. Certain parameters (the number of the interlamellar fibers and the interlamellar fibers fenestrations) were proved to have no significance in the setting of the age-dependent analysis (Table 5).

#### Influence of gender

Most of the male patients (42.9%) had the grade 3, while most of the female patients (56.3%) were in the grade 1. There was no statistically significant difference among the number of the male and female patients in different grades ( $\chi^2 = 2.162$ ,  $p = 0.339$ ).

Nevertheless, when the multifactorial analysis was applied and influence of the gender factor was tested simultaneously with the group factor, the females seemed to be more susceptible to the elastic lamellae defects since their elastic skeleton parameters indicated thinning and a statistically significant decrease in the number of the elastic lamellae and the increased size of fenestrations as confirmed by the ANOVA or Kruskal-Wallis tests with the post-hoc multiple comparison LSD test (Table 6).

Table 4

**Influence of ageing (< 65 years and ≥ 65 years) to the morphometric parameters of the elastic lamellae and interlamellar fibers**

Parameters	Mean ± SD	95% CI	<i>p</i>
Elastic lamellae			
the number			
< 65	4.05 ± 1.33	3.99–4.10	< 0.001†**
≥ 65	3.58 ± 1.54	3.52–3.65	
the thickness			
< 65	2.03 ± 0.68	2.00–2.06	< 0.001†**
≥ 65	1.88 ± 0.58	1.84–1.91	
the number of fenestrations			
< 65	1.37 ± 0.94	1.32–1.42	0.999
≥ 65	1.34 ± 0.88	1.30–1.38	
the size of fenestrations			
< 65	6.07 ± 3.88	5.90–6.24	< 0.001**
≥ 65	7.79 ± 5.16	7.57–8.01	
Interlamellar fibers			
the number			
< 65	1.37 ± 0.89	1.31–1.43	0.010**
≥ 65	1.51 ± 0.79	1.46–1.56	
the thickness			
< 65	1.18 ± 0.48	1.14–1.21	0.010†**
≥ 65	1.12 ± 0.47	1.10–1.15	
the number of fenestrations			
< 65	1.69 ± 0.98	1.59–1.79	0.009**
≥ 65	1.49 ± 0.84	1.40–1.58	
the size of fenestrations			
< 65	4.07 ± 2.09	3.91–4.24	< 0.001**
≥ 65	4.99 ± 2.52	4.77–5.22	

For abbreviations see under Tables 1 and 2.

The significant influence of ageing to all morphometric parameters of the elastic lamellae except for the number of elastic lamellae.

†ANOVA

\*\* statistically significant.

Table 5

**Morphometric parameters and their differences in the control group and the aortic stenosis group in the age-related groups**

Parameters	Aortic stenosis group vs. control group					
	GR 1 vs. control		GR 2 vs. control		GR 3 vs. control	
	< 65 years	≥ 65 years	< 65 years	≥ 65 years	< 65 years	≥ 65 years
Elastic lamellae						
the number	< 0.001**	<0.001**	0.078	<0.001**	< 0.001**	<0.001**
the thickness (μm)	< 0.001**	<0.001**	0.753	<0.001**	< 0.001**	0.017**
the number of fenestrations	0.861	0.015**	1.000	0.006**	0.469	0.922
the size of fenestrations (μm)	< 0.001**	0.932	< 0.001**	0.863	< 0.001**	0.646
Interlamellar fibers	0.090	1.000	0.414	0.998	1.000	0.923
the number						
the thickness (μm)	< 0.001**	0.011**	< 0.001**	0.233	< 0.001**	0.017**
the number of fenestrations	0.818	0.272	0.916	0.546	1.000	1.000
the size (μm)	0.066	0.006**	0.622	0.991	0.003**	0.001**

For abbreviations see under Tables 1 and 2.

Aging potentiates effects of the aortic stenosis to the elastic lamellae, but the morphometric parameters of the elastic lamellae are not entirely dependable on aging. The number of the elastic lamellae fenestrations changed intensively with aging. The size of elastic lamellae fenestrations increased significantly in the patients younger than 65 years of age. The number of the interlamellar fibers and the number of interlamellar fibers fenestrations are not changed significantly.

\*\**p* values are statistically significant.

Table 6

**Morphometric parameters in the aortic stenosis group and the control group – influence of gender**

Parameter	The aortic stenosis group	The control group	<i>p</i>	
	Mean ± SD	Mean ± SD	Gender	Groups and gender††
Elastic lamellae				
the number				
males	4.26 ± 1.44	3.24 ± 0.89	0.003†**	0.001**
females	3.11 ± 0.99	3.33 ± 0.89		
the thickness				
males	2.02 ± 0.63	1.30 ± 0.51	0.273†	0.056
females	1.94 ± 0.62	1.57 ± 0.41		
the number of fenestrations				
males	1.93 ± 1.10	1.24 ± 0.54	0.325	<0.001**
females	1.33 ± 0.55	1.58 ± 0.81		
the size of fenestrations				
males	5.11 ± 3.57	11.85 ± 8.47	0.669	0.005**
females	6.95 ± 4.75	9.36 ± 5.86		
Interlamellar fibers				
the number				
males	1.52 ± 0.80	1.43 ± 0.60	0.707	0.578
females	1.40 ± 0.81	1.45 ± 0.68		
the thickness				
males	1.11 ± 0.38	0.90 ± 0.32	0.143	0.085
females	1.33 ± 0.58	0.88 ± 0.38		
the number of fenestrations				
males	1.47 ± 0.79	1.67 ± 1.06	0.983	0.021**
females	1.79 ± 1.03	1.35 ± 0.58		
the size of fenestrations				
males	4.56 ± 2.55	5.42 ± 2.13	0.843	0.414
females	4.94 ± 2.39	5.18 ± 2.42		

For abbreviations see under Tables 1 and 2.

A statistically significant decrease of the number of the elastic lamellae and increased size of elastic lamellae fenestrations in the females with the aortic stenosis. However, the number of fenestrations is smaller.

†ANOVA test; †† the *post-hoc* multiple comparisons LSD, Kruskal-Wallis test; \*\*statistically significant.

#### *Correlation with the echocardiographic parameters*

##### *Echocardiographic parameters and grades*

The values of the echocardiographic parameters are given in Tables 7 and 8.

No statistical significance was confirmed among the echocardiographic parameters of the different grades by the ANOVA test and Bonferroni multiple comparison *post-hoc* testing (Table 7).

However, the additional testing by the Pearson's correlation test revealed a significant association of the echocardiographic parameters and aortic wall parameters and the defined grades.

The statistically significant and positive correlations were established between the SV and SVI and the tunica intima thickness and the intima-to-media ratio (Table 8), confirming that the SV diameter and SVI increase with the advanced structural changes of the aortic wall.

The AscA correlated statistically significantly and negatively with the tunica intima thickness and statistically significantly and positively to the intima-to-media ratio (Table 8), demonstrating that dilatation of the aorta was still an indicator of the aortic degeneration. The same analogy could be driven from the significant and negative association of the

AA with the wall thickness, and the significant and positive correlation of the AscA/AA pointed (Table 8).

A significant direct correlation was confirmed between the SVI and the tunica intima thickness in the grade 1 ( $R = 0.632$ ,  $p = 0.050$ ) and between the STJ and the thickness of the wall ( $R = 0.677$ ,  $p = 0.032$ ) and that of the adventitia ( $R = 0.634$ ,  $p = 0.049$ ) in the grade 2. In the grade 3, a significant direct correlation was confirmed between the STJ/AA and the wall thickness ( $R = 0.882$ ,  $p = 0.048$ ), while an indirect correlation was confirmed between the SVI and the media thickness ( $R = -0.895$ ,  $p = 0.040$ ).

##### *Influence of ageing*

Two echocardiographic parameters identified the age-related changes – STJ and STJ/AA when ANOVA was used to test the differences of the echocardiographic parameters between the age-related groups (Figure 3E). The additional multifactorial analysis obtained by the univariate analysis of variance was used to confirm the simultaneous impact of age and grade to the echocardiographic parameters (Table 7). The increase of all echocardiographic parameters with the grades was confirmed in the group of patients younger than 65 years of age (Figure 4). These differences, however, were not statistically significant, except for the AscA parameter

(Table 7). A statistically significant difference ( $p = 0.032$ ) was confirmed for the means of the largest diameter of the visualized ascending aorta (AscA) when the age factor was considered. It was  $3.56 \pm 0.16$  cm for the whole sample, but reached  $4.50 \pm 0.62$  cm in the grade 3 in the group younger than 65 years of age as a potential cut-off point for consideration (Table 7).

Also, the only parameter that was the highest in the GR 1 instead of the GR 3 was the means of the STJ/AA index in the group younger than 65 years of age. Value of this pa-

rameter for the whole sample was  $1.015 \pm 0.042$  and reached  $1.086 \pm 0.055$  in the GR 1 ( $p = 0.407$ ) (Table 7).

Higher values of the echocardiographic parameters in the patients younger than 65 years of age could be associated with more intensive derangement of some elastic lamellae features in these patients as concluded previously. Morphometry confirmed that the elastic lamellae in the patients with the aortic stenosis younger than 65 years of age were more fenestrated.

Table 7

### Echocardiographic parameters – impact of the grades and combined age and grade influence

Parameter	GR 1 (n = 14)	GR 2 (n = 6)	GR 3 (n = 10)	Mean $\pm$ SD	<i>p</i>
Age (years), means $\pm$ SD	61 $\pm$ 8.42	62.6 $\pm$ 8.38	67.5 $\pm$ 4.37	62.88 $\pm$ 7.84	0.244
Atherosclerosis, n					
none	12	6	8		
type 2 (fatty streaks) lesions	2				
type 3 (intermediate) lesions			2		
Echocardiographic parameters					
AA (cm)	2.57 $\pm$ 0.40	2.60 $\pm$ 0.29	2.63 $\pm$ 0.25	2.59 $\pm$ 0.34	0.941
SV (cm)	2.92 $\pm$ 0.52	2.84 $\pm$ 0.39	2.76 $\pm$ 0.39	2.87 $\pm$ 0.45	0.759
STJ (cm)	2.72 $\pm$ 0.54	2.56 $\pm$ 0.63	2.74 $\pm$ 0.39	2.69 $\pm$ 0.51	0.817
AscA (cm)	3.56 $\pm$ 0.72	3.38 $\pm$ 0.45	3.41 $\pm$ 0.61	3.49 $\pm$ 0.63	0.825
SVI	0.898 $\pm$ 0.168	0.874 $\pm$ 0.139	0.842 $\pm$ 0.137	0.880 $\pm$ 0.152	0.763
STJ/AA	1.066 $\pm$ 0.170	0.984 $\pm$ 0.180	1.045 $\pm$ 0.135	1.044 $\pm$ 0.161	0.642
AscA/AA	1.391 $\pm$ 0.231	1.304 $\pm$ 0.149	1.295 $\pm$ 0.164	1.351 $\pm$ 0.201	0.541
Echocardiographic parameters per age and grade					
AA (cm)					
< 65 years	2.61 $\pm$ 0.40	2.63 $\pm$ 0.38	3.10 $\pm$ 0.13	2.78 $\pm$ 0.14	0.567
$\geq$ 65 years	2.50 $\pm$ 0.45	2.55 $\pm$ 0.21	2.53 $\pm$ 0.12	2.53 $\pm$ 0.11	
SV (cm)					
< 65 years	3.03 $\pm$ 0.56	2.90 $\pm$ 0.52	3.50 $\pm$ 0.20	3.14 $\pm$ 0.18	0.490
$\geq$ 65 years	2.73 $\pm$ 0.40	2.75 $\pm$ 0.21	2.61 $\pm$ 0.17	2.70 $\pm$ 0.14	
STJ (cm)					
< 65 years	2.84 $\pm$ 0.63	2.87 $\pm$ 0.65	3.10 $\pm$ 0.26	2.93 $\pm$ 0.20	0.717
$\geq$ 65 years	2.52 $\pm$ 0.24	2.10 $\pm$ 0.14	2.67 $\pm$ 0.38	2.43 $\pm$ 0.16	
AscA (cm)					
< 65 years	3.62 $\pm$ 0.68	3.67 $\pm$ 0.32	4.50 $\pm$ 0.38	3.93 $\pm$ 0.25	§0.032**
$\geq$ 65 years	3.45 $\pm$ 0.86	2.95 $\pm$ 0.07	3.19 $\pm$ 0.33	3.20 $\pm$ 0.20	
SVI					
< 65 years	0.934 $\pm$ 0.179	0.873 $\pm$ 0.193	1.060 $\pm$ 0.024	0.956 $\pm$ 0.062	0.497
$\geq$ 65 years	0.832 $\pm$ 0.139	0.875 $\pm$ 0.050	0.798 $\pm$ 0.095	0.835 $\pm$ 0.049	
STJ/AA					
< 65 years	1.086 $\pm$ 0.149	1.083 $\pm$ 0.144	1.000 $\pm$ 0.027	1.056 $\pm$ 0.66	0.407
$\geq$ 65 years	1.030 $\pm$ 0.219	0.835 $\pm$ 0.120	1.054 $\pm$ 0.148	0.973 $\pm$ 0.052	
AscA/AA					
< 65 years	1.391 $\pm$ 0.162	1.400 $\pm$ 0.087	1.450 $\pm$ 0.043	1.414 $\pm$ 0.083	0.506
$\geq$ 65 years	1.392 $\pm$ 0.349	1.160 $\pm$ 0.071	1.264 $\pm$ 0.163	1.272 $\pm$ 0.066	

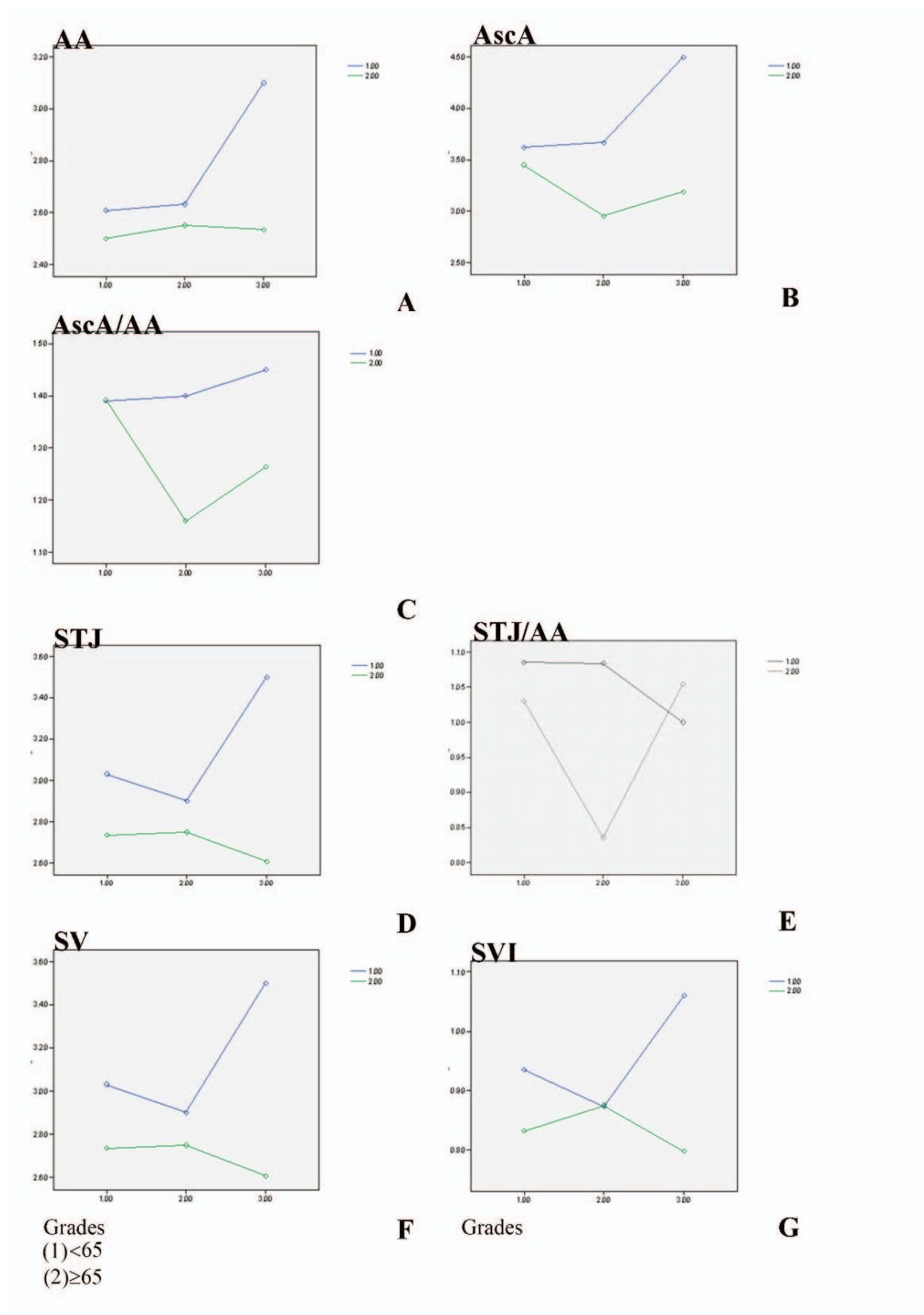
For abbreviations see under Tables 1 and 2.

A statistically significant difference was confirmed for the means of the largest diameter of the visualized ascending aorta (AscA) when the age factor was considered.

AA – diameters at the level of ventriculo-aortic junction, SV – diameters of the sinus Valsalvae, STJ – diameters at the level of the sinotubular junction, AscA – the largest diameter of the visualized ascending aorta, SVI – the sinus Valsalvae index.

*p* values obtained by ANOVA; <sup>¶</sup>*p* values obtained by the univariate analysis of variance; <sup>§</sup>statistical significance per age,

\*\**p* values are statistically significant.



**Fig. 4 – Echocardiographic parameters of the aortic stenosis group: A) AA – diameters at the level of ventriculo-aortic junction; B) AsCA – the largest diameter of visualized ascending aorta; C) AsCA/AA index; D) STJ – diameters at the level of the sinotubular junction; E) STJ/AA index; F) SV – diameters of the sinus of Valsalva; G) SVI – the sinus of Valsalva index.**

**The increase of echocardiographic parameters with grades was confirmed in a group of patients younger than 65 years (1) and in comparison to a group older than 65 years.**

Table 8

**Pearson's correlation between the echocardiographic parameters and morphometric parameters of the ascending aorta wall**

Echocardiographic parameters	The thickness of the intima	The maximal thickness of the intima	The thickness of the media	The thickness of the wall	The thickness of the adventitia	Intima-media ratio
AA	0.030	-0.382	-0.433	-0.439	-0.475	0.148
R	0.935	0.276	0.212	0.028**	0.196	0.684
<i>p</i>						
SV	0.740	0.541	-0.048	-0.121	-0.265	0.799
R	0.014**	0.106	0.894	0.757	0.491	0.006**
<i>p</i>						
STJ	0.454	-0.121	-0.050	-0.072	-0.112	0.530
R	0.187	0.738	0.890	0.855	0.774	0.115
<i>p</i>						
AscA	0.501	0.147	-0.401	-0.548	-0.385	0.695
R	0.140	0.686	0.047**	0.126	0.306	0.026**
<i>p</i>						
SVI	0.766	0.539	0.104	0.037	-0.182	0.808
R	0.010**	0.108	0.775	0.924	0.640	0.005**
<i>p</i>						
STJ/AA	0.507	0.248	0.434	0.547	0.551	0.466
R	0.135	0.489	0.210	0.005**	0.004**	0.174
<i>p</i>						
AscA/AA	0.518	0.703	-0.081	-0.031	0.039	0.600
R	0.125	0.023**	0.825	0.937	0.922	0.067
<i>p</i>						

A statistically significant and positive correlations between the SV and SVI and the tunica intima thickness and the intima-to-media ratio; a statistically significant and negative correlations of the AscA with the tunica intima thickness and a statistically significant and positive to the intima-to-media ratio; a statistically significant and negative correlation of the AA with the wall thickness; a statistically significant and positive correlation of the AscA/AA with the wall thickness.

AA – diameters at the level of ventriculo-aortic junction; SV – diameters of the sinus of Valsalva; STJ – diameters at the level of the sinotubular junction; AscA – the largest diameter of the visualized ascending aorta; SVI – the sinus of Valsalva index.

\*\*statistically significant correlation.

#### Influence of gender

Multifactorial analysis obtained by the univariate analysis of variance was used to confirm influence of the gender, age and grade to the echocardiographic parameters. A statistically significant difference was confirmed for the diameter of the SV ( $p = 0.022$ ) and a borderline significance for the mean diameter of the STJ ( $p = 0.053$ ) when gender factor was considered. No other statistical significance was confirmed by the multifactorial analysis of the gender, age and grade.

The mean diameter at the level of AA for the males was  $2.78 \pm 0.14$  cm and  $2.52 \pm 0.096$  cm for females.

The mean diameter of the SV for the males was  $3.18 \pm 0.12$  cm and  $2.70 \pm 0.11$  cm for the females. The mean diameter of the STJ for the males was  $2.98 \pm 0.19$  cm and  $2.50 \pm 0.13$  cm for the females. These parameters were proved to be gender-dependent, higher in the males and were statistically significant.

The means of the largest diameter of AscA for the males were  $3.99 \pm 0.21$  cm and for the females  $3.23 \pm 0.14$  cm. The means of the SVI for the males was  $0.970 \pm 0.057$  and  $0.833 \pm 0.038$  for the females. The means of the STJ/AA index were  $1.078 \pm 0.060$  for the males and  $1.001 \pm 0.040$  for the females. The means of the AscA/AA index were  $1.443 \pm 0.077$  for the males and  $1.286 \pm 0.052$  for the females.

#### Discussion

Treatment decisions for the ascending aorta replacement related to the aortic valve disease have not been clearly reported, because only a few studies have analyzed the evolution of the aortic wall changes in patients with the TAV stenosis. Majority of these studies considered a wide spectrum of disorders. Our study, however, focuses on a specific issue: the TAV stenosis and its influence to the aortic wall.

The results of the previous studies support one of two opposing views: one group provides evidence for the AVR without simultaneous replacement of the ascending aorta, while the other group of studies proves the attitude that the simultaneous AVR and the ascending aorta replacement is needed.

Girdauskas et al.<sup>12</sup>, using magnetic resonance imaging, found that the aortic segment in a direct contact with the systolic transvalvular flow jet was located at the greater curvature in nearly all patients and the systolic transvalvular flow jet hitting the right-lateral segment of the tubular ascending aorta was the most common scenario.

Gaudino et al.<sup>13</sup> published the results of a follow-up study of patients submitted to the AVR only and showed moderate dilatation of the ascending aorta with the expansion rate of  $0.3 \pm 0.2$  mm/year after 14.7 years postoperatively<sup>11</sup>.

Similarly, Yasuda et al.<sup>14</sup> reported a mean ascending aorta expansion rate of 0.08 mm/m<sup>2</sup>/year in a series of 14 patients followed for 9.7 years after surgery. Andrus et al.<sup>15</sup> reported results of a vast study which comprised 107 patients with an aortic diameter of  $\geq 3.5$  cm. They found no evidence of further dilation in the first 3 years after the isolated AVR. Botzenhardt et al.<sup>16</sup> even described a reduction of the aortic diameter in 10 patients with the pre-operative aortic diameter of  $\geq 4$  cm, 4.8 years after the isolated valve surgery. Gaudino et al.<sup>13</sup> and Yasuda et al.<sup>14</sup> supposed that correction of the aortic stenosis in these patients stabilized the hemodynamics and prevented further development of the aortic wall changes. Andrus et al.<sup>15</sup> concluded that in patients with the aortic valve stenosis and with the accompanying mild or moderate ascending aortic dilatation (3.5 cm to 4.9 cm) the AVR alone may be reasonable.

Ergin et al.<sup>17</sup> advocated more liberal indications for the AVR simultaneously with the ascending aorta replacement since it significantly improves the postoperative outcome in comparison to patients with the AVR and already dilated aorta.

The aforementioned controversies open the field for further investigations both in histological and clinical domain and for studies that correlate both aspects as our study.

Comparison of patients with the bicuspid and the TAV suggested that the presence of the bicuspid aortic valve induced the more severe aortic wall alterations than the degenerative changes of the tricuspid valve<sup>16</sup>. The patients with the TAV stenosis and the ascending aorta dilatation had more severe defects of the ascending aorta than the patients with the bicuspid valve and the same degree of dilatation<sup>18</sup>.

What answers has our study offered beyond the conclusions of these previous studies and to the questions that initiated our research?

Are there any changes in the basic structure of the ascending thoracic aorta due to the severe stenosis of the TAV? We proved mathematically the significant thinning of the ascending aorta wall and all of its tunics in the patients with the aortic stenosis. Similar changes were already described in a different model of exaggerated hemodynamic forces and its influence on the arterial wall<sup>19</sup>.

Is there a gradual progression of the aortic wall changes caused by the aortic stenosis and could we establish a grading system for these changes or apply the already existing ones? The results of our study clearly demonstrated three histopathological GRs with a mathematical distinction to the controls and among different GRs. The grading system of Schlattmann and Becker<sup>8</sup> was applicable. Furthermore, our supposition is that these three GRs follow the natural progression and evolution of the aortic stenosis and its hemodynamic impact to the aortic wall. The elastic lamellae and interlamellar fibers generally became thinner and fragmented. The number of the lamellae decreased statistically significantly with the GR, while the number of the interlamellar fibers significantly increased. There was a principle in spatial distribution of these changes in the aortic wall as they affected the internal media first. These observations are in line with the previous similar studies<sup>8, 20, 21</sup>.

Could we find some irreversible changes in the wall of the ascending aorta in the patients with the severe aortic stenosis, but with no dilated aorta? We consider that the grade 3 with the destructive changes in the numerous elastic lamellae and disorganization of the smooth muscles resembled the irreversible changes.

Could we distinguish the ageing-induced changes from the aortic stenosis induced changes? This is quite a peculiar question. The morphological and morphometric characteristics of the elastic skeleton are generally changed during ageing which makes the arteries prone to different influences, including the hemodynamics. Even the "perfect" internal thoracic artery is prone to the elastic skeleton changes induced by ageing<sup>8</sup>. Nakashima et al.<sup>20</sup> proved that the number of the elastic lamellae fenestrations increased with ageing, as did the number of the interlamellar elastic fibers, their ramifications and the number of their fenestrations.

All analyzed parameters in our study changed statistically significantly with ageing. It was very important to prove that the observed GRs are not merely effects of ageing. We proved that described changes persisted in both groups of patients, the younger and older than 65 years of age, they were potentiated with ageing, but they were not entirely the effect of ageing. The elastic skeleton decay during ageing in the patients with the aortic stenosis could be explained by the synergistic effects of ageing and the aortic stenosis to the aortic wall as well as by a prolonged duration of the aortic stenosis in the group older than 65 years of age.

Female gender proved to be associated with more intensive changes in the aortic stenosis as compared to the control group. It seems that the aortic stenosis and female gender act in the same direction to the histopathological changes of the elastic lamellae and that the aorta of females is more sensitive to different hemodynamic influences as already proved for the abdominal aortic aneurysms model<sup>11</sup>. On the opposite, in the bicuspid aortopathy, the female gender is proved to have protective effect<sup>22</sup>.

Finally, could we correlate the histological grades with the echocardiographic parameters? The aortic valve disease is associated with the ascending aortic dilatation because of "hemodynamic burdens caused by forceful jets"<sup>23, 24</sup>. Supposingly, a size of the dilatation is related to the degree of turbulence induced by the stenotic valve and the severity of stenosis<sup>25</sup>. However, Linhartova et al.<sup>1</sup> concluded that there was no independent association between the severity of aortic stenosis and the aortic diameter, indicating that factors other than the aortic stenosis itself could affect the echocardiographic parameters of the aorta. They indicated that the geometry of the aortic orifice, its influence to the flow distribution pattern and histopathological changes in the aortic wall deserved to be investigated further.

Our study proved a significant correlation of the SV and SVI parameters with the thickness of the intima and the intima-to-media ratio and indicated that the SV diameter and index increased with the structural changes of the aortic wall. The SV was also higher in the male patients.

Despite the open controversies about the ascending aorta diameter and its association with the actual structural

changes of the aorta, there was a statistically significant correlation of the AscA with the morphological parameters of the aorta wall disruption. This parameter was particularly important since it was shown that its value of 4.5 cm could be predictive for the irreversible defects especially in the patients younger than 65 years of age.

Beside that, two more parameters, the STJ and STJ/AA, correlated well with the age related changes and morphometric parameters in the GRs 2 and 3. The STJ was also higher in the male patients.

However, the grades could not be associated with simple diameters measured by echocardiography and them tested by the ANOVA analysis.

Many other authors concluded, also, that "size is not enough"<sup>23,24</sup>. These studies implicate that other parameters are necessary for the estimation of the histopathological defects and the severity of the aortic stenosis.

#### Limitations of the study

Our study considers a relatively small number of the patients. However, due to a nature of the morphometric research, it gives a global and correct insight into the status of the elastic skeleton of the ascending aorta in patients with degenerative stenosis of the TAV.

#### Conclusion

Our findings strongly support the view that the aortas of patients with the TAV stenosis are submitted to the hemody-

namic stress which subsequently leads to the gradual elastic lamellae disruption. The exact region of the hemodynamic influence could be confirmed morphometrically. Changes of the elastic skeleton were potentiated with ageing. Females were submitted to more intensive disruption of the elastic lamellae through the course of the aortic stenosis.

The changes of the aortic wall statistically significantly correlated with the echocardiographic parameters: AA, SV, AscA and SVI. The echocardiographic parameters tended to be higher in the grade 3 in patients younger than 65 years of age. The AscA value of more than 4.5 cm was associated with the irreversible morphological defects in these patients. The SV and STJ were higher in the male patients.

Our study is in accordance with opinions that the ascending aorta replacement should be considered in relation to the AVR, particularly in patients younger than 65 years of age with the ascending aortas of more than 4.5 cm in a diameter and the TAV stenosis.

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## Relation between resilience and cigarette/alcohol use in adolescents with mild intellectual disability

Odnos između rezilijentnosti i upotrebe cigareta i alkohola kod adolescenata sa intelektualnom ometenošću

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### Abstract

**Background/Aim.** Resilience is related to the substance use in adolescence. However, little is known about the nature of this relation in adolescents with intellectual disability (ID). The aim of this research was to determine the relation among three domains of resilience (sense of mastery, sense of relatedness and emotional reactivity) and the substance use (cigarettes, alcohol, and marijuana) in adolescents with ID. **Methods.** The sample consisted of 100 adolescents of both genders with mild ID and aged 13–20 years. The Resiliency Scales for Children and Adolescents were used to assess resilience. **Results.** In the adolescents with ID, resilience was significantly related to the use of cigarettes ( $\chi^2 = 13.384$ ;  $df = 3$ ;  $p = 0.004$ ), but not to the use of alcohol ( $\chi^2 = 6.789$ ;  $df = 3$ ;  $p > 0.05$ ). Out of the three assessed domains of resilience, increased emotional reactivity was the only significant predictor of cigarette use. **Conclusion.** The obtained results suggest that emotional difficulties may increase the risk of cigarette use in adolescents with ID.

### Key words:

adolescent; alcohol drinking; intellectual disability; resilience, psychological; smoking.

### Apstrakt

**Uvod/Cilj.** Rezilijentnost je povezana sa upotrebom psihoaktivnih supstanci u adolescenciji, ali malo se zna o prirodi ove veze kod adolescenata sa intelektualnom ometenošću (IO). Cilj istraživanja bio je utvrđivanje povezanosti između tri domena rezilijentnosti (osećanja kontrole nad vlastitim životom, osećanja povezanosti i emocionalne reaktivnosti) i upotrebe cigareta, alkohola i marihuane kod adolescenata sa IO. **Metode.** Uzorak je činilo 100 adolescenata sa IO, oba pola, uzrasta 13–20 godina. Za procenu rezilijentnosti korišćene su Skale rezilijentnosti za decu i adolescente (*Resiliency Scales for Children and Adolescents*). **Rezultati.** Kod adolescenata sa IO rezilijentnost je bila značajno povezana sa upotrebom cigareta ( $\chi^2 = 13,384$ ;  $df = 3$ ;  $p = 0,004$ ), ali ne i sa upotrebom alkohola ( $\chi^2 = 6,789$ ;  $df = 3$ ;  $p > 0,05$ ). Od tri ispitana domena rezilijentnosti, samo je povišena emocionalna reaktivnost bila značajan prediktor upotrebe cigareta. **Zaključak.** Dobijeni rezultati sugerišu da emocionalne teškoće mogu povećati rizik od upotrebe cigareta kod adolescenata sa IO.

### Ključne reči:

adolescenti; alkohol, pijenje; intelektualna ometenost; rezilijentnost, psihološka; pušenje.

### Introduction

The study of resilience has a relatively long history which began by identifying the characteristics of resilient children, i.e., the characteristics which can account for individual differences in withstanding and recovering from stressful situations<sup>1–3</sup>. The concept of resilience developed over time, and the attention of researchers was also being directed to understanding the processes which lead to the successful adaptation despite adversity and traumas<sup>4–5</sup>. Contemporary literature provides an even broader

definition of resilience: “the capacity of dynamic system to withstand or recover from significant threats to its stability, viability or development”<sup>6</sup>. Regardless of the described changes in the conceptualization of resilience, the interest in individual characteristics which contribute to positive developmental outcomes has been sustained up to the present time. According to the authors who follow this course of research, resilience “embodies the personal qualities that enable one to thrive in the face of adversity”<sup>7</sup>. Resilience is believed to be a multidimensional construct which includes some characteristics of temperament

and personality, and also specific skills which enable an individual to successfully overcome life difficulties<sup>8</sup>.

The main focus of this paper are domains of resilience distinguished and described by Prince-Embury<sup>9</sup>: a sense of mastery, sense of relatedness, and emotional reactivity. Sense of mastery includes three individual qualities: optimism as a positive attitude towards the world, one's own life and the future, self-efficacy, i.e., confidence in one's own abilities, and adaptability which involves openness to criticism and the ability to learn from one's own mistakes. Individual qualities included in the sense of relatedness are: a sense of trust, perceived access to support, comfort with others and tolerance of differences. Emotional reactivity includes sensitivity, i.e., speed and intensity of an emotional response and two constructs which represent the outcomes of emotional regulation – recovery and impairment after emotional excitement.

Research on the relation between thus conceptualized resilience and the substance use (SU) in the general population of adolescents reveals that sense of mastery and sense of relatedness negatively correlate, while emotional reactivity positively correlates with the SU<sup>10</sup>. Apart from that, literature on the role of described individual qualities of resilience in the occurrence and the development of the SU in typically developing adolescents is extensive. With regard to the sense of mastery, research results suggest that the SU is related to a lower level of self-efficacy<sup>11–13</sup>, optimism<sup>14–16</sup> and adaptability<sup>17–19</sup>. The results of studies on the second domain of resilience, the sense of relatedness, indicate that relationship with parents negatively correlates with the SU<sup>20–22</sup>, but that relationship with peers may have the opposite effect<sup>23–25</sup>. Finally, numerous authors associate the SU with emotional reactivity, i.e., with difficulties in experiencing and regulating emotions<sup>26–28</sup>.

The relation between resilience and the SU in the general population of adolescents has been well researched. However, little attention was given to studying this relation in adolescents with intellectual disability (ID). On the other hand, the results of previous studies have confirmed that many adolescents with ID have experience with the use of cigarettes, alcohol and marijuana.

Some authors found that the prevalence of cigarette use in adolescents with ID is lower compared to the general population<sup>29–30</sup>. By contrast, the results of some studies showed a higher prevalence of cigarette use in adolescent with ID<sup>31–32</sup> or absence of significant differences when compared to the general population<sup>33–34</sup>. Empirical data on the proportion of adolescents with ID who tried cigarettes are not consistent: 3.4%<sup>35</sup>; 16% of boys and 17% of girls<sup>31</sup>; 30.1%<sup>36</sup>; 59.5%<sup>29</sup>. The assessments of the incidence of smoking also differ: 4.9%–26.9% are currently smoking<sup>30</sup>, i.e., 30%<sup>34</sup>; 15% smoked more than once, and 14% are currently smoking<sup>32</sup>; 27% of boys and 21% of girls smoked during the previous year<sup>31</sup>; 1.4% smoke regularly<sup>29</sup>.

Older studies reported that the prevalence of alcohol was lower in adolescents with ID than in the general population<sup>30, 37</sup>. However, significant differences related to that were not found in more recent studies<sup>29, 33–34</sup>. The literature provides the following data on the incidence of trying alco-

hol in adolescents with ID: 41%<sup>32</sup>; 48%<sup>37</sup>; 71.7%<sup>29</sup>. Authors who have dealt with these problems have different observations about the incidence of alcohol use: 22.7%–54.5% consumed alcohol in the previous year<sup>30</sup>, i.e., 29.5%<sup>36</sup>; 8.8%–35.5% consumed alcohol in the previous month<sup>30</sup>, i.e., 39%<sup>37</sup>; 0.6% drink alcohol regularly<sup>35</sup>.

It is generally believed that the use of illegal drugs is less prevalent in adolescents with ID than in the general population<sup>29, 33–34, 37</sup>. However, the research results indicate that a significant number of adolescents with ID tried marijuana: 10%<sup>34</sup>; 13%<sup>33, 37</sup>; 34.3%<sup>29</sup>. Marijuana was used by 0.9%–13.8%<sup>30</sup>, i.e., 10% of adolescents<sup>37</sup> over the previous month, and 1.5%–23.9% during the previous year<sup>30</sup>.

The aim of this study was to determine the existence and nature of the relations among three domains of resilience (sense of mastery, sense of relatedness, and emotional reactivity) and cigarette, alcohol, and marijuana use in adolescents with ID. With regard to the previous studies, it was assumed that the SU negatively correlated with the sense of mastery and positively with emotional reactivity. However, inconsistent findings on the role of a sense of relatedness did not provide a basis for making initial assumptions about the relation between this domain of resilience and the SU.

## Methods

The research was conducted in four schools for students with disabilities in Belgrade. The sample included 100 adolescents with ID, aged 13–20 years [average age: (mean  $\pm$  standard deviation (SD)) = 15.59  $\pm$  1.736 years], of both genders (63% boys and 37% girls). There were no significant differences in the average age of the male and female participants ( $t = 0.574$ ;  $df = 98$ ;  $p > 0.05$ ). The participants' intellectual functioning was at the level of mild ID (IQ = 50–69). The sample included only the adolescents with adequate verbal abilities who were assessed as being able to give answers on a Likert-type scale. The sample did not include the adolescents with dual diagnoses and multiple disabilities.

The data on the participants' age, gender, intellectual functioning, and health were taken from the school records.

The Peabody Picture Vocabulary Scale (PPVT-IV)<sup>38</sup> was used for the assessment of participants' verbal abilities. Form A was applied in this research, with 114 items divided into 16 sets which test the knowledge of nouns, verbs, and adjectives from 20 different areas (e.g., plants and professions).

The Youth Risk Behavior Survey (YRBS)<sup>39</sup> was used to collect data on the SU. Only the questions from the Scale on the SU related to the history of cigarette use (eight questions), alcohol use (six questions), and marijuana use (four questions) were used in this research. Due to the considerable differences in the responses to questions about the incidence of usage, data on a whole-life prevalence of cigarette, alcohol, and marijuana use were used in the research, and the participants were grouped with regard to whether they had ever used the given psychoactive substances or not. Internal consistency of the scale applied in this research was good ( $\alpha = 0.862$ ).

The Resiliency Scales for Children and Adolescents (RSCA)<sup>9</sup> were used to assess resilience. The instrument consisted of 64 questions distributed in the following three scales: the Sense of Mastery (MAS) scale consists of Optimism, Self-efficacy and Adaptability subscales; the Sense of Relatedness (REL) scale consists of the Sense of Trust, Perceived Access to Support, Comfort with Others and Tolerance of Differences subscales; the Emotional Reactivity (REA) scale consists of Sensitivity, Recovery and Impairment subscales. The higher scores on the MAS and REL scales and the lower scores on the REA scale point to greater resilience. Internal consistency of the RSCA ( $\alpha = 0.894$ ) as well as the MAS ( $\alpha = 0.820$ ), the REL ( $\alpha = 0.880$ ) and the REA ( $\alpha = 0.924$ ) scales was good in this research.

The informed consent was obtained from the school, parents and participants for the purpose of this research. Class teachers selected students with adequate verbal abilities who were able to participate in the research. Also, before giving out the questionnaires, the assessment of receptive speech was conducted by means of the PPVT-IV. The participants achieved standard scores in the range 94–185 (mean  $\pm$  SD =  $135.61 \pm 22.861$ ). The research aims were explained and instructions on data collecting procedure were given to each participant. The participants were informed that participation in the research was voluntary and that their responses were confidential. The questionnaires were completed in a separate room in the school, without the presence of anybody else but the examiner and participants. The questions were read as they were given in questionnaires, with necessary additional explanations. The participants were required to choose one of the given answers. Cards with provided answers were made in order to make it easier for the participants to answer the questions.

Descriptive statistics, correlation method, and regression analysis (binary logistic analysis) were used in data analysis.

## Results

Out of 100 participants, a total of 49% reported cigarette use, 63% reported alcohol use and 4% reported marijuana use at least once in their lifetime.

Table 1 includes data on the range, mean, and SD of the raw scores for the MAS, REL, and REA scales and their subscales.

**Table 1**  
**Descriptive measures of scores on the Resiliency Scales for Children and Adolescents (RSCA)**

RSCA scales and subscales	Range min–max	Mean $\pm$ SD
MAS	31–73	50.83 $\pm$ 8.016
optimism	10–25	17.37 $\pm$ 3.569
self-efficacy	9–36	23.98 $\pm$ 5.077
adaptability	1–12	9.48 $\pm$ 1.801
REL	40–96	71.34 $\pm$ 10.050
sense of trust	10–28	20.65 $\pm$ 3.273
perceived access to support	10–24	19.64 $\pm$ 2.830
comfort with others	6–16	11.46 $\pm$ 2.298
tolerance of differences	10–28	19.59 $\pm$ 3.822
REA	0–66	33.17 $\pm$ 13.761
sensitivity	0–22	11.94 $\pm$ 4.397
recovery	0–16	5.41 $\pm$ 3.015
impairment	0–38	15.82 $\pm$ 8.402

**MAS – mastery; REL – relatedness; REA – reactivity; SD – standard deviation; min – minimum; max – maximum.**

The relation between the main variables was tested by means of the correlation method. Table 2 shows the values of the Pearson's correlation coefficient. Cigarette use had a statistically significant positive correlation with the scores on the Adaptability subscale, the REA scale and its subscales – Sensitivity, Recovery and Impairment. Alcohol use had a statistically significant positive correlation with the scores on the REA scale and its Sensitivity and Impairment subscales and a negative correlation with the scores on the Optimism subscale. There were no significant correlations between marijuana use and the scores on the Resiliency Scales for Children and Adolescents.

The relation between resilience and cigarette/alcohol use was assessed by a series of binary logistic analyses. The binary logistic analyses were not performed for marijuana use, since very few of the participants stated that they had tried marijuana (4 out of 100 participants) and there were no significant correlations.

**Table 2**  
**Correlations between scores on the Resiliency Scales for Children and Adolescents (RSCA) and cigarette, alcohol and marijuana use**

RSCA scales and subscales	Cigarettes	Alcohol	Marijuana
MAS	0.028	-0.105	0.171
optimism	-0.085	-0.200*	0.180
self-efficacy	0.032	-0.007	0.102
adaptability	0.206*	-0.049	0.116
REL	-0.043	-0.024	0.044
sense of trust	0.030	0.000	0.053
perceived access to support	0.019	-0.120	0.026
comfort with others	0.074	0.136	0.071
tolerance of differences	-0.147	-0.055	0.009
REA	0.352 <sup>†</sup>	0.218*	0.027
sensitivity	0.292 <sup>†</sup>	0.198*	0.003
recovery	0.233*	0.132	0.040
impairment	0.339 <sup>†</sup>	0.206*	0.029

\* $p < 0.05$ ; <sup>†</sup> $p < 0.01$ .

For other abbreviations see under Table 1.

Table 3

## Characteristics of the resilience dimensions as predictors in regression model

RSCA scales	B	S.E.	Wald	df	<i>p</i>	Exp(B)
MAS	0.002	0.030	0.007	1	0.936	1.002
REL	-0.010	0.024	0.177	1	0.674	0.990
REA	0.059	0.018	10.741	1	0.001	1.060

For abbreviations see under Table 1.

The results of binary logistic analyses indicated that participants' resilience was related to cigarette use ( $\chi^2 = 13.384$ ;  $df = 3$ ;  $p = 0.004$ ), with the achieved scores on REA scale being singled out as the only significant predictor (Table 3). By contrast, the assessed dimensions of resilience (MAS, REL, and REA) were not significant predictors of alcohol use ( $\chi^2 = 6.789$ ;  $df = 3$ ;  $p > 0.05$ ).

The second series of binary logistic analyses assessed the predictive value of scores achieved on the subscales of MAS (Optimism, Self-efficacy and Adaptability), the subscales of REL scale (Sense of Trust, Perceived Access to Support, Comfort with Others and Tolerance of Differences) and the subscales of REA scale (Sensitivity, Recovery and Impairment). The achieved scores on the mentioned subscales were not the significant predictors either of cigarette use ( $\chi^2 = 17.955$ ;  $df = 10$ ;  $p = 0.056$ ) or alcohol use ( $\chi^2 = 13.841$ ;  $df = 10$ ;  $p = 0.180$ ).

## Discussion

Although the prevalence of the SU in adolescents with ID is not the subject of this study, the obtained results deserve a brief comment. The incidence of cigarette and alcohol use was higher when compared to the results of other studies conducted on the samples of adolescents with ID in the USA<sup>30</sup>, Great Britain<sup>31–32, 34, 36</sup> and Taiwan<sup>35</sup> and lower when compared to the empirical data from South Africa<sup>29</sup>. However, the incidence of marijuana use was significantly lower when compared to the results of studies conducted in other countries<sup>29–30, 33–34, 37</sup>.

This research analyzed the relation among three domains of resilience and cigarette, alcohol, and marijuana use in the adolescents with ID. Compared to the normative population, 9 the participants' scores on the MAS scale were below the average range, in the average range on the REL scale and above the average range on the REA scale. The most important findings indicated that the sense of mastery and sense of relatedness were not significantly related to the SU while emotional reactivity was.

Contrary to our expectations, a negative correlation between the sense of mastery and the SU was not confirmed. A very low positive correlation was determined between Adaptability and cigarette use. This finding may be compared to observations of other authors who found that, in the population of people with ID, smoking had a higher incidence in those with developed adaptive skills<sup>40–42</sup>, and that cigarette use was a symbol of maturity and competence<sup>43</sup>, or a means to blend in<sup>44</sup>. Also, a very low negative correlation was determined between Optimism and alcohol use, which is in accordance with the results of the previously mentioned

studies indicating that adolescents who had more positive expectations of the future used alcohol less frequently<sup>14–16</sup>. When interpreting the results on the relation between sense of mastery and the SU, we should bear in mind that the participants generally had the scores below average on the MAS scale. Thus, it is possible that the applied instrument was not sensitive enough to detect subtle individual differences in the adolescents with ID.

The absence of significant correlations between the sense of relatedness and the SU was somewhat expected. As already mentioned, the results of the previous studies suggested that the nature of this relation varied depending on whether the relationship was with parents or with peers. The questions referring to the relationships with parents and peers were not separated in the REL scale which could have influenced the obtained results.

The results of this research indicated that emotional reactivity was a significant predictor of cigarette use in the adolescents with ID. In generally sparse literature on the SU in adolescents with ID, the studies on the relation between the SU and emotional difficulties are quite rare. However, although scarce, the existing studies on the risk factors of the SU in the adolescents with ID suggest that the mental health problems have a particularly important role<sup>36, 45–47</sup>.

The obtained results are in accordance with the results of numerous studies which point to a significant relation between the SU and emotional difficulties in the general population of adolescents. The authors who researched the relation between negative emotions and the SU were consistently finding that adolescents with higher negative affectivity used substances more frequently to overcome, or alleviate unpleasant emotions<sup>28, 48–49</sup>. The conclusions of the study which summarized the results of relevant research in this field emphasized that apart from negative affectivity, higher positive affectivity and poor regulation of emotions also had a significant role in initiation of the SU<sup>50</sup>. In other words, intensive emotional states, oversensitivity to emotional stimuli, and inability to control emotions increased the risk of the SU in adolescence. Empirical data on frequent co-morbidity of the SU and depression and anxiety disorders in adolescence<sup>26, 51–52</sup> should also be mentioned. The literature related to this subject shows that the prevalence of co-morbidity of the SU and depression ranges from 11.1% to 32%, and of the SU and anxiety disorders from 7% to 40.4%<sup>53</sup> in adolescents.

According to the results of this research, emotional reactivity was significantly related to cigarette use, but not to alcohol use. A stronger connection of the emotional difficulties with cigarette use than with alcohol use was also confirmed in the studies conducted on samples of typically developing adolescents<sup>26</sup> and adolescents with ID<sup>32</sup>.

The relation between cigarette use and emotional difficulties in the general population of adolescents has a good empirical basis, and research results on this subject were summed up in several reviews of the relevant literature<sup>54-56</sup>. A common conclusion of these studies is that emotional difficulties increase the risk of cigarette use in adolescence. The results of this research are supported by the empirical data on a higher incidence of cigarette use in adolescents with ID and the mental health problems<sup>32</sup>.

The relation between emotional difficulties and the SU can be explained by self-medication hypothesis which implies that the SU functions as a compensatory means for modulating negative emotions and alleviating unpleasant psychological states<sup>57</sup>. According to this hypothesis, the choice of psychoactive substances depends on the type of internal difficulties a person feels and physiological and psychological effects of the given substances. With regard to cigarette use, adolescents usually justify smoking by sedative and anxiolytic effects of nicotine, and suggest that the need for relaxation and stress reduction are their motives for cigarette use<sup>55</sup>. The observation of some authors is particularly interesting. According to them, the effects which adolescents experience when first consuming cigarettes influence their smoking later in a way that regular smoking is more frequent in those who experience more positive and fewer negative effects<sup>58</sup>.

An explanation of the obtained results may also be sought in the motivational model of alcohol use in adolescence, according to which one of the motives is a desire or a need to avoid or alleviate unpleasant emotional experiences<sup>59</sup>. This motive is considered to be an intermediary in the relation between negative emotions and the SU<sup>28</sup>. In this case, the SU represents a reactive process preceded by experiencing negative emotions and may be related to the symptoms of anxiety<sup>60</sup> and depression<sup>49</sup>. A similar pattern was determined in one of the few studies on how to cope with the stress strategies in persons with ID who used substances<sup>61</sup>. The authors of this research reported that persons with ID who used substances were prone to a "palliative" pattern of reacting to stress, i.e., that they had poorer skills of relaxing and engaging in other activities when they were in stressful situations. The research in which the mentioned motivational model was applied to cigarette use showed that adolescents with increased anxiety were particularly prone to cigarette use, motivated by avoiding unpleasant emotions because of anxiolytic effects of nicotine<sup>60</sup>.

The influence of emotional difficulties on the SU varies depending on the presence of other risk factors. With regard to that, special attention was given to the influence of peer groups and empirical findings confirmed that the relation between negative emotions and the SU increased by socializing with peers who used psychoactive substances<sup>27</sup>. The SU is more available in such an environment, while alternative activities and interactions which may help overcome negative emotions are highly limited<sup>48</sup>. Although this research did not include the characteristics of friendly relationships, it is reasonable to assume that education in a restrictive environment, consisting exclusively of peers with ID, does not provide enough possibilities to learn effective emotion regulating strategies.

The literature discusses the possibility that emotional difficulties and the SU have a common etiology in genetic influences and environmental factors<sup>62</sup>. This viewpoint is supported by empirical data on higher incidence of the SU in adolescents with a family history of substance abuse<sup>50</sup>.

Finally, many authors believe that the relation between the SU and emotional difficulties is two-way, i.e., that the emotional difficulties may have a role of a risk factor or a consequence, depending on the phase of using psychoactive substances. The increased level of the emotional difficulties increases the risk of initiation and experimental use of psychoactive substances, but the regular SU contributes to emotional difficulties<sup>50</sup>. Cigarette use may contribute to an increased level of stress and negative affectivity<sup>54-56</sup> and proved to be a more significant predictor of severe symptoms of depression in adolescents than alcohol use<sup>62, 63</sup>.

This study has certain limitation that need to be mentioned. First of all, attention was directed to the individual qualities which represented only one aspect of a complex resilience construct. Future research should be aimed at environmental influences and the interaction of the individual and environmental factors. Second, only data obtained from the adolescents with ID were used. Thus, different sources of information, methods and instruments should be used for assessing resilience and the SU in the future. Third, the research does offer a possibility to draw conclusions on causal relations between the assessed variables which points to the need for longitudinal research of this subject.

Despite the mentioned limitations, the findings of this study provided a significant insight into insufficiently researched relation between the SU and a sense of mastery, sense of relatedness, and emotional reactivity in adolescents with ID.

Identification of factors which contribute to the SU in adolescence has important implications for prevention. The results of this research suggest that the attention should be directed to the emotional difficulties which increase the risk of SU in adolescents with ID. Alleviating emotional difficulties may be a strong motivating factor for the SU and may increase adolescents' susceptibility to negative influences of peers and the media. Therefore, in preventing the SU in adolescents with ID, priority should be given to interventions aimed at improving self-control and emotion regulation as well as learning effective strategies to overcome negative emotions and stress. Bearing in mind the data on the incidence of cigarette and alcohol use in adolescents with ID, these interventions should be applied to a universal level, through organized curricular and extracurricular activities for all students.

## Conclusion

The results of this research may contribute to understanding the relation between individual qualities of resilience and the SU in adolescents with ID. The obtained findings with a significant predictive value of emotional reactivity complement the extensive literature on the relation between the emotional difficulties and the SU in adolescence. Future research should be aimed at studying mechanisms underlying this relation.

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## Relation between osteocalcin and the energy metabolism in obesity

### Povezanost osteokalcina i energetskeg metabolizma kod gojaznosti

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#### Abstract

**Background/Aim.** Numerous findings have indicated the potential relation between the osteocalcin, the traditional parameter of bone turnover and the regulation of energy metabolism. The aim of this study was to identify the relationship between osteocalcin and calculated indexes, which evaluate insulin sensitivity, insulin resistance and/or secretory capacity of the pancreas, in non-diabetic, obese subjects. **Methods.** The study included 57 (11 men and 46 women) euglycemic, obese patients (the body mass index – BMI:  $41.03 \pm 6.61$  kg/m<sup>2</sup>) and 48 healthy individuals, age and sex matched (BMI:  $23.15 \pm 2.04$  kg/m<sup>2</sup>). Plasma glucose and the insulin levels during the two-hour oral glucose tolerance test (OGTT) were determined in order to calculate the Homeostatic Model Assessment (HOMA) indexes (HOMA-IR, HOMA-B%), EISI (estimated insulin sensitivity index), EFP (estimated first phase) and ESP (estimated second phase). Osteocalcin was measured by using the Electro-chemiluminescence (ECLIA) methodology. **Results.** Statistically lower osteocalcin was found in

the obese subjects ( $24.72 \pm 9.80$  vs  $33.31 \pm 10.89$  ng/mL;  $p < 0.01$ ). There was a statistically significant positive correlation between osteocalcin and EISI ( $r = 0.340$ ;  $p < 0.01$ ). The inverse correlations were found between the osteocalcin and HOMA-IR ( $r = -0.276$ ;  $p < 0.01$ ), HOMA-B% ( $r = -0.337$ ;  $p < 0.01$ ), EFP ( $r = -0.332$ ;  $p < 0.01$ ) and ESP ( $r = -0.266$ ;  $p < 0.01$ ). Multiple regression showed that the BMI and osteocalcin have a significant inverse prediction with the EISI and HOMA-IR, but the level of prediction of the BMI was substantially higher. **Conclusion.** The effect of osteocalcin in the glycoregulation is evident, but its contribution is significantly smaller in relation to other obesity associated factors. Therefore, when assessing its position and the role in glycemic control it is always necessary to bear in mind that osteocalcin represents only one of the many contributing factors, some of which exhibit dominant influence than osteocalcin itself.

#### Key words:

obesity; insulin resistance; osteocalcin; pancreas; body mass index.

#### Apstrakt

**Uvod/Cilj.** Brojna dosadašnja saznanja ukazala su na postojanje uloge osteokalcina, tradicionalnog parametra metaboličke aktivnosti kosti, u regulaciji metabolizma ugljenih hidrata. Cilj ove studije bio je da se utvrdi postojanje relacije između osteokalcina i izračunatih parametara procene stepena insulinske osetljivosti/rezistencije i sekretorne sposobnosti pankreasa kod gojaznih, nedijabetičnih ispitanika. **Metode.** Studijom je obuhvaćeno 57 gojaznih osoba (11 muškaraca i 46 žena) (indeks telesne mase – ITM:  $41,03 \pm 6,61$  kg/m<sup>2</sup>) i 48 zdravih, normalno uhranjenih ljudi (ITM:  $23,15 \pm 2,04$  kg/m<sup>2</sup>) koji odgovaraju ispitivanoj grupi, po starosti i polu. Svim ispitanicima izmerena je glukoza i insulin u toku dvočasovnog testa oralnog opterećenja glukozom (OGTT), osteokalcin i izračunate vrednosti *Homeostatic Model Assessment* (HOMA) indeksa (HOMA-IR, HOMA-B%), EISI (indeksa insulinske osetljivosti), (*estimated first phase*) i

EFP (*estimated second phase*) druge faze (ESP) i procena sekretarnog kapaciteta pankreasnih ćelija druge faze (ESP). Koncentracija osteokalcina merena je u serumu, elektrohemiluminescentnom (ECLIA) metodologijom. **Rezultati.** Utvrđene su statistički značajno niže vrednosti osteokalcina u grupi gojaznih (ITM =  $24,72 \pm 9,80$  vs  $33,31 \pm 10,89$  ng/mL;  $p < 0,01$ ). Linearnom korelacionom analizom dobijen je visok stepen pozitivne povezanosti osteokalcina sa EISI ( $r = 0,340$ ;  $p < 0,01$ ). Inverzna korelacija utvrđena je između osteokalcina i HOMA-IR ( $r = -0,276$ ;  $p < 0,01$ ), HOMA-B% ( $r = -0,337$ ;  $p < 0,01$ ), EFP ( $r = -0,332$ ;  $p < 0,01$ ) i ESP ( $r = -0,266$ ;  $p < 0,01$ ). ITM i osteokalcin imaju značajnu inverznu predikciju sa EISI i HOMA-IR, ali je nivo predikcije ITM bitno viši u odnosu na osteokalcin. **Zaključak.** Uticaj osteokalcina na sistem glikoregulacije je evidentan, ali je njegov udeo u tome bitno manji u odnosu na gojaznost i druge činioce povezane sa gojaznošću. Zbog toga se pri proceni mesta i uloge osteokalcina u glikoregulaciji, uvek mora imati u vidu da je osteokalcin,

kao sistemski medijator, samo jedan od brojnih drugih faktora, pri čemu neki od njih, ispoljavaju dominantnije uticaje od osteokalcina.

**Ključne reči:**

**gojaznost; insulin, rezistencija; osteokalcin; pankreas; telesna masa; indeks.**

## Introduction

Obesity is defined as a condition of increasing of fat in total body weight and as such represents an important risk factor for the development of many diseases which are based on insulin resistance<sup>1, 2</sup>. Dysfunctional adipose tissue has a high degree of metabolic activity, producing a wide range of humoral mediators that are closely related to the level of bone metabolic activity. Based on these findings, in the early 21st century, a hypothesis was made – obesity affects the metabolic activity of the bones, and that feedback, bone tissue through humoral factors such as osteocalcin may regulate some aspects of glycemic control<sup>3, 4</sup>.

Osteocalcin is one of the most important noncollagenous proteins that participates in the process of bone mineralization. Based on studies on animal models, osteocalcin released in circulation has a prominent role in glycemic control through two main mechanisms: by acting directly on the islet cells of Langerhans, increasing the production and secretion of insulin<sup>5</sup> and by acting through the indirect mechanisms, primarily through adiponectin<sup>6, 7</sup> on insulin-sensitive cells of the peripheral tissues (muscle and adipose tissue)<sup>8, 9</sup>.

This was followed by clinical studies that examined the relationship between osteocalcin and traditional parameters which evaluate glucose metabolism. For the most part, studies were conducted on subjects with already altered glycemic control, with the values of glycemia over the euglycemic range<sup>10</sup>.

In accordance with these facts, this study focuses on the analysis and testing of relations between osteocalcin as a traditional marker of the bone metabolism and the standard laboratory markers of the glucose metabolism.

## Methods

This cross-sectional study was conducted in the Department of Endocrine Diagnostics in cooperation with the Outpatient Department of Clinic for Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Vojvodina, during 2015. The study included 57 obese patients (11 men and 46 women) and 48 healthy, normal weight subjects which correspond to the study group by age and gender.

Criteria for exclusion from the study were: disorders of glycemic control (elevated fasting glucose, impaired glucose tolerance and/or diabetes mellitus), endocrinologic diseases, liver diseases which exclude steatohepatitis and include hepatic steatosis), kidney diseases, psychic disorders and the presence of metabolic bone diseases as well as supplementation with vitamin D and/ or calcium preparations.

## Anthropometric measurements

To all subjects the body height (BH) and body weight (BW), were measured. The body height was measured by using the Anthropometer according to Martin and is expressed in centimeters (cm); and the BW was determined on the decimal scale in kilograms (kg). The body mass index (BMI) was calculated by using the formula:  $BMI (kg/m^2) = BW (kg) / BH^2 (m^2)$  in order to determine the presence of obesity ( $BMI > 30 kg/m^2$ )<sup>11</sup>.

## Oral glucose tolerance test

After an eight-hour, overnight fasting, standard oral glucose tolerance test (OGTT) was performed to all subjects in order to measure the glucose and insulin plasma levels in basal condition as well as in 120th minute after the oral administration of 75 g of glucose. Blood was taken by venipuncture, using the Vacuette® system. The hemolytic, lipemic and icteric samples were not taken for the analysis. For determination of osteocalcin, the obtained serum samples were frozen at -20 °C and stored until moment of the analysis. All other laboratory parameters were determined directly after obtaining the serum and plasma samples.

## Determination of glucose, insulin and glycated hemoglobin A1c

Glucose was measured by the biochemical method (hexokinase) on the Abbott Architect c8000 analyzer by using the commercial kits from the same manufacturer. The reference value for glucose was from 4.1 to 6.1 mmol/L. Insulin was determined by the direct Chemiluminiscence technology (CLIA) on the automated system ADVIA Centaur XP. The recommended reference value for insulin in basal conditions is from 3.0 to 25.0 mIU/L. Glycated hemoglobin A1c (HbA1c) was measured by using the immunoturbidimetric method of inhibition of agglutination on microparticles, on the automated system Abbott Architect ci 4100.

## Calculating indexes for the evaluation of glycoregulation

In order to evaluate insulin sensitivity, the EISI (estimated insulin sensitivity index) was calculated to all subjects. On that occasion the following formula was used<sup>12</sup>:  $EISI = 0.222 - 0.00333 \times BMI - 0.0000779 \times Ins_{120} - 0.000422 \times age$

where Ins = insulin

As part of the assessment of the secretory capacity of pancreatic beta cells, EFP (estimated first phase) and ESP

(estimated second phase) were calculated by using following formulas <sup>12</sup>:

$$\text{EFP} = 2.032 + 4.681 \times \text{Ins0} -$$

$$135.0 \times \text{Gluc120} + 0.995 \times \text{Ins120} + 27.99 \times \text{BMI} -$$

$$269.1 \times \text{Gluc0}$$

$$\text{ESP} = 277 + 0.800 \times \text{Ins0} -$$

$$42.79 \times \text{Gluc120} + 0.321 \times \text{Ins120} + 5.338 \times \text{BMI}$$

were Ins = insulin and gluc = glucose

Also, the Homeostatic model assessment (HOMA) indexes (HOMA-IR and HOMA-B%) were determined to all participants by using HOMA 2 calculator. The calculator was downloaded from the official website of the Oxford School of Medicine <sup>13</sup>, based on the measured values of the blood glucose and insulin levels in basal conditions. A cut-off value for the HOMA-IR is defined as a value less than 2.5 <sup>14</sup>.

#### *Determination of osteocalcin and crosslaps levels*

The total osteocalcin (Osteo) was determined on the automated system, Cobas e 411 Roche Diagnostics. The lower limit of detection was 0.5 ng/mL. The osteocalcin values were expressed in ng/mL, and according to the manufacturer, the following reference values were defined – for women: 11–48 ng/mL, and for men: 14–46 ng/mL). The crosslaps was determined on the automated Cobas s 411 system (the reference value of women's reproductive period: 299–573 pg/mL; for men: 300–704 pg/mL).

#### *Statistical analysis*

Data are presented using the descriptive statistical methods, continuous variables as mean  $\pm$  standard deviation (SD). In order to evaluate the differences between the groups we used the Mann-Whitney U test. The correlations among variables were assessed by the Pearson's correlation

coefficient. The multiple regression analysis was performed to assess the independent association between the BMI, osteocalcin levels and calculated parameters of insulin resistance/sensitivity as well as insulin secretion. A 2-tailed  $p < 0.05$  was considered statistically significant. Statistical analysis was performed using the Data Analysis Excel (Microsoft Corp., Redmond, WA) and MedCalc 12.1.4.0 statistical software (MedCalc Software, Mariakerke, Belgium).

## **Results**

The examined group consisted of 57 obese subjects (46 females and 11 males) (BMI:  $41.03 \pm 6.61$  kg/m<sup>2</sup>). Compared to the control group, the obese subjects differed significantly in all monitored parameters except the blood glucose values in basal, as well as in 120th minute of the standard OGT test ( $p = 0.689$ ;  $p = 0.714$ ). Based on the results, the study group had significantly lower osteocalcin levels ( $p < 0.01$ ) (Table 1).

The linear correlation analysis revealed a significant degree of positive correlation between the osteocalcin levels and the calculated EISI ( $r = 0.340$ ;  $p < 0.01$ ). An inverse correlation was found between osteocalcin and the HOMA-IR ( $r = -0.276$ ;  $p < 0.01$ ), the HOMA-B% ( $r = -0.337$ ;  $p < 0.01$ ), EFP ( $r = -0.332$ ;  $p < 0.01$ ) and the ESP ( $r = -0.266$ ;  $p < 0.01$ ) (Table 2). There was no significant correlation between the osteocalcin and plasma insulin ( $r = -0.165$ ;  $p > 0.05$ ) and glucose levels ( $r = -0.007$ ;  $p > 0.05$ ) after 120 minute of the OGTT as well as with basal glycaemia ( $r = 0.137$ ;  $p > 0.05$ ) while a very low degree of correlation was present with basal insulin ( $r = -0.288$ ;  $p < 0.01$ ). The BMI exhibited a significant correlation with all the examined parameters, but all levels of the correlation were higher than one with osteocalcin. The BMI had a negative correlation only with the EISI ( $r = -0.998$ ;  $p < 0.01$ ) (Table 2).

**Table 1**

**The significance of differences between the examined and control group**

Parameters	Obese (n = 57) mean $\pm$ SD	Control (n = 48) mean $\pm$ SD	<i>p</i>
BMI (kg/m <sup>2</sup> )	41.03 $\pm$ 6.61	23.15 $\pm$ 2.04	< 0.01
Gluc 0 (mmol/L)	4.94 $\pm$ 1.04	4.75 $\pm$ 0.44	0.238
Gluc120 (mmol/L)	5.44 $\pm$ 1.83	5.02 $\pm$ 1.17	0.172
Ins 0 (mIU/L)	16.86 $\pm$ 9.36	6.15 $\pm$ 3.75	< 0.01
Ins120 (mIU/L)	36.93 $\pm$ 26.11	14.32 $\pm$ 11.44	< 0.01
HbA1c (%)	5.67 $\pm$ 0.79	5.08 $\pm$ 0.38	< 0.01
Osteo (ng/mL)	24.72 $\pm$ 9.81	33.31 $\pm$ 10.89	< 0.01
CsIs (pg/mL)	384.40 $\pm$ 190.87	500.90 $\pm$ 207.51	0.003
EISI	0.07 $\pm$ 0.02	0.13 $\pm$ 0.01	< 0.01
HOMA-IR	2.38 $\pm$ 1.29	0.89 $\pm$ 0.54	< 0.01
HOMA-B (%)	186.46 $\pm$ 82.45	97.18 $\pm$ 34.84	< 0.01
EFP	1231.51 $\pm$ 441.81	766.67 $\pm$ 219.89	< 0.01
ESF	289.39 $\pm$ 76.13	195.12 $\pm$ 49.59	< 0.01

**Legend:** Gluc 0 and gluc 120 – plasma glucose values in 0 and 120 the minute of Oral glucose tolerance test (OGTT); Ins 0 and ins 120 – plasma insulin levels in 0 and 120 minute of OGTT; HbA1c – glycated haemoglobin A1c; Osteo - osteocalcin; CsIs – crosslaps; 25OHD – vitamin D; EISI – estimated insulin sensitivity index, calculated according to Stumvoll et al. <sup>12</sup>; EFP – estimated first phase, calculated according to Stumvoll et al. <sup>12</sup>; ESP – estimated second phase. calculated according to Stumvoll et al. <sup>12</sup>; Homeostatic Model Assessment (HOMA)-IR – HOMA index for estimation of the insulin resistance, using HOMA 2 calculator; HOMA-B% – HOMA index for estimation of insulin secretion, using HOMA 2 calculator; *p* – statistical significance.

Table 2

Linear correlation analysis					
Parameteres	Osteo (ng/mL)		Parameteres	BMI (kg/m <sup>2</sup> )	
	r	p		r	p
BMI (kg/m <sup>2</sup> )	-0.335	< 0.01	Osteo (ng/mL)	-0.335	< 0.01
Gluc 0 (mmol/L)	0.137	> 0.05	Gluc 0 (mmol/L)	0.259	< 0.01
Gluc 120 (mmol/L)	-0.007	> 0.05	Gluc 120 (mmol/L)	0.242	< 0.01
Ins 0 (mU/mL)	-0.288	< 0.01	Ins0 (mU/mL)	0.680	< 0.01
Ins 120 (mU/mL)	-0.165	> 0.05	Ins120 (mU/mL)	0.505	< 0.01
EISI	0.340	< 0.01	EISI	-0.998	< 0.01
HOMA-IR	-0.276	< 0.01	HOMA-IR	0.691	< 0.01
HOMA-B%	-0.337	< 0.01	HOMA-B%	0.558	< 0.01
ESP	-0.266	< 0.01	ESP	0.591	< 0.01
EFP	-0.332	< 0.01	EFP	0.516	< 0.01

**Legend:** Gluc 0 and gluc 120 – plasma glucose values in 0 and 120 the minute of Oral glucose tolerance test (OGTT); Ins 0 and ins 120 – plasma insulin levels in 0 and 120 minute of OGTT; HbA1c – glycated haemoglobin A1c; Osteo – osteocalcin; Csls – crosslaps; 25OHD – vitamin D; EISI – estimated insulin sensitivity index, calculated according to Stumvoll et al. <sup>12</sup>; EFP – estimated first phase, calculated according to Stumvoll et al. <sup>12</sup>; ESP – estimated second phase, calculated according to Stumvoll et al. <sup>12</sup>; Homeostatic Model Assessment (HOMA)-IR – HOMA index for estimation of the insulin resistance, using HOMA 2 calculator; HOMA-B% – HOMA index for estimation of insulin secretion, using HOMA 2 calculator; p – statistical significance.

Table 3

Multiple regression analysis						
Model	Dependent variable	Adjusted R <sup>2</sup>	p	Predictors		
					t	p (t)
A	EISI	0.862	< 0.001	BMI	-11.321	< 0.001
				Osteo	5.207	< 0.001
B	HOMA-IR	0.798	< 0.001	BMI	13.435	< 0.001
				Osteo	-3.109	0.002
C	HOMA-B%	0.835	< 0.001	BMI	13.329	< 0.001
				Osteo	-1.102	0.272
D	EFP	0.874	< 0.001	BMI	14.068	< 0.001
				Osteo	0.655	0.513
E	ESP	0.920	< 0.001	BMI	17.073	< 0.001
				Osteo	1.607	0.361

**Legend:** Adjusted R<sup>2</sup> – coefficient of determination that is compliant with the number of independent variables included in the model; p – statistical significance; EISI – estimated insulin sensitivity index; HOMA – homeostatic model assessment; EFP – estimated first phase; ESP – estimated second phase; BMI – body mass index; Osteo – osteocalcin.

The multiple regression analysis was used in order to examine an independent predictive potential of osteocalcin in relation to the individual parameters of glycemic control, in all subjects (Table 3). In the model A (R<sup>2</sup> = 0.862,  $p < 0.001$ ), we observed that the serum levels of osteocalcin, positively and independently contribute to the EISI values. Also, the model B (R<sup>2</sup> = 0.798,  $p < 0.001$ ) showed that the serum levels of osteocalcin negatively and independently contribute to the HOMA-IR. In addition to a statistically significant predictive impact of osteocalcin on the EISI and HOMA-IR (models A and B), *t* coefficients indicated that the influence of the BMI to specified indexes was more pronounced than of osteocalcin itself. Osteocalcin, in the models C, D and E did not show a significant predictive potential in relation to the HOMA-B%, EFP and ESP.

## Discussion

The examined group consisted of 57 obese patients (obesity grade II and grade III). In comparison to the healthy controls, the group of obese subjects did not differ significantly in the measured

values of the plasma glucose levels during two-hour OGTT. However, the statistically significant elevated HbA1c values may indicate the presence of the modified discrete regime of glycemic control in the group of obese patients.

Our results are in agreement with the known facts that obese subjects compared to normally weighted, ones have decreased insulin sensitivity and/or the increased insulin resistance <sup>15</sup>. According to the obtained results, the examined group had significantly higher levels of insulin in basal conditions and after 120 minutes of the OGT Test, which could be responsible for maintaining the plasma glucose levels in the reference range.

Also, the calculated index that assesses the insulin resistance (HOMA-IR) was significantly higher in the obese, while the indicator of insulin sensitivity (EISI) was significantly lower in the obese compared to the control group. The aforementioned index is widely used in everyday clinical practice and represents a measure of quantifying insulin resistance and insulin sensitivity.

Our analysis showed that obesity had a very high degree of correlation with the parameters of insulin

secretion: the HOMA-B%, EFP and ESP. Due to the fact, obesity is essentially connected to an increased insulin resistance and elevation of insulin secretion reflects the strong compensatory mechanism in order to maintain normoglycemia. The obtained results indicate that in obesity, with clearly altered biological effects of insulin on the effector cells, functioning capacity of the islet cells of Langerhans is completely preserved, and operates in the mode of hypersecretion. Since among the examined group, hiperglycemia or decreased glucose tolerance was not registered, this group of obese patients was characterized by an initial, the mildest form of abnormal glucose regulation.

The obese patients showed statistically lower values of osteocalcin, compared to the control group of healthy subjects. Similar results were obtained by Cifuentes et al.<sup>16</sup> Lucey et al.<sup>17</sup> showed a significantly lower value of the total osteocalcin in the patients with the BMI value equal or lower than to 34.9 kg/m<sup>2</sup> in comparison to women of normal weight.

Apart from the already mentioned fact that obese people have lower levels of osteocalcin, we found a significant negative correlation between the BMI and osteocalcin levels. It is believed that osteocalcin released from the bone tissue to the circulation is becoming a mediator who stimulates the proliferation of pancreatic beta cells and insulin secretion, and on the other hand increases the insulin sensitivity of peripheral tissue<sup>18–20</sup>. The mechanism which can explain this relation is an abnormal secretion of leptin in obesity which may decrease bone metabolic activity, primarily through the central, sympathetic regulation of bone cells. It is also known that leptin decreases the expression of adiponectin and its concentration in circulation. In mice, the parenteral administration of osteocalcin increases the expression of adiponectin in white adipose tissue due to the improvement of insulin sensitivity, reduction of triglycerides in the liver and muscles as well as the inhibition of gluconeogenesis<sup>21–23</sup>.

Osteocalcin shows a highly significant correlation with the EISI and HOMA-IR which make it one of indisputable regulators of the biological effects of insulin on the cell level. In respect of osteocalcin, obesity exerts the opposite effects in terms of the increased insulin resistance and decreased insulin sensitivity. Also, it can be seen that compared to osteocalcin, obesity has a significantly higher level of association with the insulin resistance and/or sensitivity. These results indicate that obesity is predominantly related to the increased insulin resistance and reduced insulin sensitivity, while the preferred level of osteocalcin connection to the manifestation of the biological insulin effects is substantially lower. The current level of the increased insulin resistance and reduced sensitivity in the group of the obese, is predominantly caused by the adverse effects of obesity in opposite to the protective effects of osteocalcin.

At first glance, the correlation results a great surprise are, which showed significant inverse relationship between osteocalcin and the already mentioned indicators of insulin secretion (HOMA-B%, EFP, ESP). These results are in stark contrast to the so far known facts suggesting that osteocalcin stimulates beta cells of Langerhans and insulin secretion<sup>24</sup>. This mechanism is mediated by the action of osteocalcin *via* the Gprc6a receptors by stimulating the proliferation of beta cells. Also, it is known that through the same receptor at the level of the gastrointestinal tract, osteocalcin stimulates the production of GLP-1 (glucagon like peptide-1) and in that way participate in the preservation of insulin secretion<sup>7, 25</sup>. The explanation for the obtained relation can be found in the fact that the study was conducted among obese people with the minimal disturbances of the glycemic control and subsequent insulin hypersecretion. Logical processing of the data shows: the greater obesity, the lower osteocalcin, and higher insulin secretion. On the basis of this fact, it can be concluded that between osteocalcin and insulin secretion there is an inverse correlation.

In order to comprehensively examine the influence of different factors on the insulin secretion in obese subjects, the multiple linear regression analysis was performed. It is necessary to bear in mind that the factor of obesity is complex and comprises a plurality of factors that directly or indirectly influence both insulin secretion and insulin resistance/sensitivity. This statistical method showed that osteocalcin has no significant prediction of insulin secretion (the HOMA-B%, EFP, ESP), opposite to the complex factor of obesity (BMI). Therefore, it could be concluded that the chosen model of this research, although conducted on the small sample size, is not suitable for studying the influence of osteocalcin on insulin secretion.

### Conclusion

The obtained significantly lower concentrations of osteocalcin in the obese subjects, compared with those of normal weight, are the consequences of the altered energy metabolism in obesity.

The inverse relationship between osteocalcin and obesity indicate an actual connection between the bone metabolism and glycemic control in the complex system of the energy metabolism.

The impact of osteocalcin on glycemic control is evident, but its share is substantially lower in relation to obesity and other factors associated with obesity. Therefore, when assessing the place and role of osteocalcin in the glycemic control, there should always be taken into account that osteocalcin, as a systemic mediator, is only one of many other, less influential factors.

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## Physiological adaptations following a four-week of high-intensity functional training

Fiziološka adaptacija za vreme funkcionalnog treninga visokog intenziteta u trajanju od četiri nedelje

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### Abstract

**Background/Aim.** High-Intensity Functional Training (HIIFT) is a popular mixed-modal program that utilizes both resistance and aerobic based exercises. The aim of this study was to examine the physiological effects of the HIIFT programming on physically active men (10) and women (10) over a four-week period through the measure of the aerobic capacity, anaerobic capacity, and maximal weight lifted.

**Methods.** The participants first completed a maximal oxygen consumption (VO<sub>2</sub>max) test. After 48-hours of rest, the subjects completed the anaerobic capacity test via the Wingate protocol. Following the Wingate test, the subjects performed a 1-repetition maximum test for squat, snatch and clean at the offsite training location. After the pre-measurements were obtained, the subjects entered a four-week the HIIFT intervention and returned to the lab for all post-measurements.

**Results.** Significant improvements were observed in male and female: VO<sub>2</sub> max (Pre: 46.7 ± 2.6, 33.7 ± 1.7 mL/kg/min; Post: 49.0 ± 3.0, 35.0 ± 1.8 mL/kg/min), Peak Wingate Power (Pre: 1206 ± 106, 708 ± 44 W; Post: 1283 ± 88, 809 ± 38 W) Mean Wingate Power (Pre: 680 ± 46, 704 ± 48 W; Post: 434 ± 15, 458 ± 18 W;  $p < 0.05$ ), back squat (Pre: 128.8 ± 8.8 kg, 44.1 ± 6.8 kg; Post: 142.7 ± 9.8, 54.3 ± 6.2 kg) clean (Pre: 82.5 ± 6.2, 24.1 ± 3.4 kg; Post: 92.7 ± 5.8, 33.2 ± 3.3 kg) and snatch (Pre: 59.3 ± 4.4, 20.9 ± 1.7 kg; Post: 69.1 ± 5.3, 25.0 ± 2.3 kg;  $p < 0.05$ ), respectively. No gender influence on interaction was observed over time ( $p > 0.05$ ). **Conclusion.** The HIIFT demonstrated the rapid physiological improvements in strength, aerobic and the anaerobic capacity following a four-week intervention in the physically active participants.

### Key words:

exercise; exercise test; adaptation, physiological; oxygen consumption.

### Apstrakt

**Uvod/Cilj.** Funkcionalni trening visokog intenziteta (FTVI) je popularni program mešanih modusa koji koristi i vežbe otpora i aerobne vežbe. Cilj studije bio je ispitivanje fizioloških efekata FTVI programa na fizički aktivne muškarce (10) i žene (10) tokom perioda od četiri nedelje merenjem aerobnog kapaciteta, anaerobnog kapaciteta i maksimalne podignute težine. **Metode.** Učesnici u studiji prvo su završili test maksimalne potrošnje kiseonika (VO<sub>2</sub>). Nakon odmora od 48 sati, učesnici su završili test anaerobnog kapaciteta po Wingate protokolu. Po istom protokolu učesnici su uradili test sa jednim maksimalnim ponavljanjem za čučanj, podizanje terata do nivoa deltoidnih mišića i izbačaj naglim trzajem na drugoj lokaciji za trening. Nakon prethodnih merenja, učesnici su pristupili FTVI testu u trajanju od četiri nedelje, nakon čega su urađena ponovna merenja. **Rezultati.** Značajno poboljšanje VO<sub>2</sub> max ustanovljeno je i kod muškarada i kod žena (pre: 46,7 ± 2,6, 33,7 ± 1,7 mL/kg/min; posle: 49,0 ± 3,0, 35,0 ± 1,8 mL/kg/min), Maksimalna Wingate snaga (pre: 1206 ± 106, 708 ± 44 W; posle: 1283 ± 88, 809 ± 38 W); Srednja Wingate snaga (pre: 680 ± 46, 704 ± 48 W; posle: 434 ± 15, 458 ± 18 W;  $p < 0,05$ ). Zadnji čučanj (pre: 128,8 ± 8,8 kg, 44,1 ± 6,8 kg; posle: 142,7 ± 9,8, 54,3 ± 6,2 kg). Podizanje tereta do nivoa deltoida (pre: 82,5 ± 6,2, 24,1 ± 3,4 kg; posle: 92,7 ± 5,8, 33,2 ± 3,3 kg) i izbačaj naglim trzajem: (pre: 59,3 ± 4,4, 20,9 ± 1,7 kg; posle: 69,1 ± 5,3, 25,0 ± 2,3 kg;  $p < 0,05$ ). Nije ustanovljena interakcija uticaja pola tokom vremena ( $p > 0,05$ ). **Zaključak.** FTVI je pokazao brzo fiziološko poboljšanje u snazi, aerobni i anaerobni kapacitet tokom četiri nedelje kod fizički aktivnih učesnika.

### Ključne reči:

vežbanje; vežbanje, testovi; adaptacija, fiziološka; kiseonik, potrošnja.

## Introduction

In recent years there has been a growing interest towards the non-traditional exercise programs that involve the high-intensity bouts and require a lower time commitment. Additionally, these programs do not focus on specific aspects of fitness, but rather on a broad focus towards the general preparedness. The aforementioned programs are commonly referred to as High-Intensity Functional Training (HIFT). These include the programs like Insanity, P90x®, and CrossFit®. The unique approach to the HIFT programming is the various uses of exercise modalities, ranging from Olympic weightlifting, to the bodyweight calisthenics/gymnastic movements, with an emphasis on improving the capacity to perform large amounts of work in short periods of time. However, despite the recent trends in popularity toward HIFT like training<sup>1</sup> there are little evidence regarding the efficacy of this application on markers of fitness and performance in currently active individuals.

Various acute adaptations occur during the onset of a new exercise program when applied to untrained individuals. Alterations in strength can occur as early as two weeks<sup>2</sup> while alterations in the aerobic capacity (i.e.  $\text{VO}_{2\text{max}}$ ) can occur as rapidly as six days<sup>3</sup>. This is commonly reported to be due to alterations in the neuromuscular system as well as oxygen related alterations in cells<sup>2,4</sup>. However, when beginning a new exercise program in individuals who are already physically active, alterations and adaptations occur less rapidly. Due to the reduction in adaptation following chronic training, research continually examines the efficacy of the exercise programs over longer durations (i.e., 8, 12, 16-weeks). Several studies examining this time period have shown that both aerobic and strength based interventions result in positive alterations in body composition<sup>5,6</sup> while the improvements in performance markers such as muscular strength, anaerobic and the aerobic capacity also occur in the variously trained populations<sup>7-9</sup>.

Limited information is available on the acute adaptations to a new exercise program for individuals who are already physically active. Furthermore, the advocates of the HIFT programs purport gains and early physiological adaptations for those who participate<sup>9,10</sup>. However, the acute adaptations to HIFT training have yet to be reported for individuals who are already physically active. The acute responses to HIFT in the physically active population would be beneficial for those prescribing this style in order to better assess and track improvements in clients and participants. Therefore, the purpose of this study was to determine the changes in the aerobic capacity, anaerobic capacity and 1-repetition maximum (RM) in back squat, clean and snatch, following the four-week HIFT program in physically active men and women.

## Methods

### Procedures

All participants reported to the Exercise Physiology Laboratory for the pre and post intervention testing, which

consisted of two laboratory visits 48-hours apart. During the first visit, each subject signed the informed consent and completed a health history questionnaire. The participants then completed a maximal oxygen consumption ( $\text{VO}_{2\text{max}}$ ) exercise test on a treadmill using a graded exercise protocol. The participants returned to the lab 48-hours later for the anaerobic capacity measures via the 30-second Wingate protocol. Following this measure, the participants made an appointment at a local CrossFit® affiliate and began their four-week intervention. During the first week of the acclimation period, the participants were taught a proper exercise technique and then performed a 1-RM test for squat, snatch and clean at the CrossFit® affiliate prior to the start of the four-week intervention. Following the four-week intervention, the participants returned to the laboratory for the aerobic and the anaerobic capacity testing and to the affiliate for the 1-RM testing.

### HIFT intervention

The HIFT intervention chosen for this study was CrossFit®. Following the first two lab visits (i.e., after the aerobic and anaerobic capacity testing), the participants made an appointment at a local CrossFit® box affiliate where they would be instructed by a certified instructor and exercise science graduate. All participants began an introductory week to learn the basics of HIFT. The participants were provided with a full group class schedule for the next four weeks. The participants were informed they could attend as many classes per week with no restrictions, however a minimum of three days per week was required in order to remain in the study. The participants trained with the normal HIFT classes performed the workouts as prescribed by the head trainer of the affiliate. A typical class would start with a warm-up, a strength/power component, then a HIFT bout ranging from 7–25 minutes in duration. The participants were instructed and encouraged to modify each workout as needed. Following the four-weeks of training, each subject performed the 1-RM tests again under the same supervision as well as the  $\text{VO}_2\text{max}$ , and Wingate testing, with each bout separated by at least 48 hours.

### Participants

Twenty participants (10 male, 10 female) were recruited for the current study. The participants' characteristics are shown in Table 1.

**Table 1**

**Subject characteristics**

Parameters	Male	Female
Number	10	10
Age (years), mean $\pm$ SD	26.6 $\pm$ 1.9	28 $\pm$ 2.0
Height (cm), mean $\pm$ SD	178 $\pm$ 2.3	167.5 $\pm$ 1.5
Weight (kg), mean $\pm$ SD	90.5 $\pm$ 5.8	72.2 $\pm$ 4.8
% Body Fat, mean $\pm$ SD	17.7 $\pm$ 2.0	23.7 $\pm$ 2.1

**SD – Standard Deviation.**

All participants were classified as recreationally trained by self-identifying as currently participating in at least 30-bouts of the planned physical activity for at least 30-minutes three times per week for at least the last 90-days. Additionally, all participants needed to participate both in the aerobic and weight training activities. None of the participants had the previous HIFT experience or reported any colds, sicknesses, or orthopedic conditions that could limit exercise participation. This study was approved the Institutional Review Board of the host university prior to recruitment and data collection.

#### *Maximal exercise capacity, strength and anthropomorphic measurements*

Aerobic capacity was determined through a graded exercise test; expired gases were analyzed by the True 1 analyzing system calibrated to known gases. Heart rate (Polar monitors) was obtained during the test. Each participant performed a 3–5 minute warm-up at a self-selected speed. The participants then breathed through a one-way valve, which enabled expired gases to be processed by the True 1 analyzing system. The participants started the test at a self-selected running speed at 0% grade. The grade was increased 2% every 2-minutes until the  $\text{VO}_2$  leveled off or the subject stopped the test (8–10 minutes)<sup>11</sup>. All participants attained their age predicted maximal heart rate (HR)  $\pm 10$  bpm, reported Ratings of Perceived Exertion (RPE)  $> 19$  on the Borg scale during the last stage of the test and had the Respiratory Exchange Ratio (RER)  $> 1.10$ .

The anaerobic capacity was obtained approximately 48-hours following the first visit. For the testing, the participants returned to the laboratory to have their peak and mean power measured using the Wingate test. Briefly, using the Lode cycle ergometer, the subjects pedaled at a self-selected moderate intensity for 5-minutes as a warm-up. Then, the participants performed the Wingate test against resistance equal to 7.5% of their bodyweight on a electronically controlled braking bike and had both their mean and peak power measured.

Muscular strength assessment occurred after a one-week introduction to a proper form and techniques in HIFT. After the one-week introduction, the subjects performed a

1-RM in the following order on the same day: snatch, clean and back squat. The progression was self-selected and the participants were allowed unlimited attempts at every weight and they achieved their 1-RM within 4 attempts, with no time limit provided. Each of the attempts were supervised by the certified HIFT instructor as well as the Principal Investigator (PI), a certified strength and conditioning specialist (CSCS).

#### *Statistical analysis*

All data were analyzed by the SPSS version 24 (IBM, New York, NY). Prior to the data analysis, the variables were analyzed by the Shapiro-Wilk test in order to determine normality of the data distribution. The effect of the intervention was determined using a repeated measure  $2 \times 2$  ANOVA (time by gender) and a statistical significance was set at  $p < 0.05$ . If there was an interaction effect, the Scheffe post-hoc test was utilized to identify differences.

#### **Results**

All 20 participants completed the study. Compliance was 100%, as all subjects attended the four-week classes for a minimum of three times a week. There were no injuries reported to the investigators, or to the trainers. The Shapiro-Wilk test demonstrated that the sprint data was normally distributed for all conditions. Following the 4 weeks of training, all measured variables showed the significant main effects in both genders. Table 2 shows the laboratory measures. The participant's  $\text{VO}_2$  max demonstrated a main time effect ( $p = 0.016$ ) and no time  $\times$  gender effect ( $p = 0.415$ ). The Peak Wingate Power test demonstrated the main time effect ( $p = 0.010$ ) and no time  $\times$  gender effect ( $p = 0.712$ ). The Mean Wingate Power demonstrated a main time effect ( $p = 0.026$ ) and no time  $\times$  gender effect ( $p > 0.999$ ). Table 3 shows the 1 RM measures. The participant's back squat demonstrated a main time effect ( $p < 0.001$ ) and no time  $\times$  gender effect ( $p = 0.327$ ). The participant's clean demonstrated a main time effect ( $p < 0.001$ ) and no time  $\times$  gender effect ( $p = 0.648$ ). The participant's snatch demonstrated a main time effect ( $p < 0.001$ ) and a main time  $\times$  gender effect ( $p = 0.042$ ).

**Table 2**

#### **Laboratory Performance: Aerobic & Anaerobic**

Test	Male	Confidence interval	<i>p</i> value	Effect size	Female	Confidence interval	<i>p</i> value	Effect size
$\text{VO}_2$ Max Pre (mL/kg/min)	46.7 $\pm$ 2.6	$\pm$ 1.61			33.7 $\pm$ 1.7	$\pm$ 1.05		
$\text{VO}_2$ Max Post (mL/kg/min)	49.0 $\pm$ 3.0*	$\pm$ 1.86	0.032	-0.2419	35.0 $\pm$ 1.8	$\pm$ 1.12	0.239	0.3480
Wingate Peak Power Pre (W)	1206 $\pm$ 106	$\pm$ 65.7			708 $\pm$ 44	$\pm$ 27.27		
Wingate Peak Power Post (W)	1283 $\pm$ 88*	$\pm$ 54.54	0.015	-0.3675	809 $\pm$ 38*	$\pm$ 23.55	0.025	-0.7755
Wingate Mean Power Pre (W)	680 $\pm$ 46	$\pm$ 28.51			434 $\pm$ 15	$\pm$ 29.75		
Wingate Mean Power Post (W)	704 $\pm$ 48*	$\pm$ 9.3	0.018	+0.9634	458 $\pm$ 18*	$\pm$ 11.16	0.047	+0.9592

Results are given as mean  $\pm$  standard deviation.

\* significant time main effect;  $\text{VO}_2$  – maximum oxygen consumption.

Table 3

## Laboratory Performance: Strength

Test	Male	Confidence interval	<i>p</i> value	Effect size	Female	Confidence interval	<i>p</i> value	Effect size
Back Squat Pre (kg)	128.8 ± 8.8	± 5.45			44.1 ± 6.8	± 4.21		
Back Squat Post (kg)	142.7 ± 9.8*	± 6.07	0.0006	-0.5980	54.3 ± 6.2*	± 3.84	0.008	-0.6168
Clean Pre (kg)	82.5 ± 6.2	± 3.84			24.1 ± 3.4	± 2.11		
Clean Post (kg)	92.7 ± 5.8*	± 3.59	0.0002	-0.6474	33.2 ± 3.3*	± 2.05	0.0007	-0.8052
Snatch Pre (kg)	59.3 ± 4.4	± 2.73			20.9 ± 1.7	± 1.05		
Snatch Post (kg)	69.1 ± 5.3*#	± 3.28	0.002	-0.7092	25.0 ± 2.3*#	± 1.43	0.004	-0.7118

Results are given as mean ± standard deviation.

\*significant time main effect; # main effect for time × gender.

## Discussion

The purpose of this study was to determine the changes in the aerobic capacity, anaerobic capacity and 1-RM in back squat, clean and snatch following a four-week HIFT program of the physically active men and women. The primary findings demonstrated the time-dependent improvements in the aerobic capacity, anaerobic capacity, squat, clean and snatch. A gender-by-time effect was only observed in the pre and post snatch measures. Further points of consideration are provided below.

### Markers of strength

The markers of muscular strength examined in this study were the Squat, Clean and Snatch. Following the HIFT intervention, the significant strength adaptations in each marker occurred, suggesting that the four-week time period was sufficient enough to elicit changes in the physically active participants. Acute strength adaptations are common among sedentary individuals beginning a new exercise program due to neural adaptations (e.g., synchronization and recruitment of additional motor units, increased neural drive, etc.). However, these adaptations are less observed in those who are already physically active. For instance, Ahtiainen et al.<sup>7</sup> examined the difference in strength adaptation between the trained and untrained participants where the significant alterations were observed only at 14 weeks and 10 weeks later than the observation made in the current study. It is important to note that even though the participants in the current study were physically active, their experience and technique associated with the examined markers (i.e., power clean and snatch) were limited and therefore a likely mechanism for the observed improvements following the four-week intervention.

Neuromuscular adaptation is commonly attributed to the early improvements in strength<sup>2</sup> and is greatly related to the learning and coordination of the muscle groups as it relates to the specificity of training<sup>12</sup>. This notion is supported by a 60-day unilateral strength training study performed by Narici et al.<sup>13</sup>, which yielded an approximate 9% of the improvement in the maximal voluntary contraction with no improvements in the cross-sectional area; however, an approximate 25% of the improvement in the neuromuscular activity (via the integrated electromyographic activity) occurred. An alternative explanation for the observed improvements in

strength within this study is early skeletal muscle hypertrophy. This is a rare occurrence; however, there are a few documented cases of the early adaptive hypertrophy. For example, the significant gains in strength and hypertrophy were observed in the recreationally trained participants by the third week of a 35-day high-intensity resistance training study<sup>14</sup>. It is important to note that the examination of the hypertrophic response to training is outside the scope of this study, but the notion presents an alternative explanation for the significant gains in strength observed during the four-week training period.

### Aerobic performance

The HIFT style programming is not traditionally prescribed for the improvements in the aerobic performance. However, a recent research has shown increases in the oxygen consumption following a high-intensity training<sup>15</sup>, and therefore, garnered more interest in the scientific community. For instance, Helgerud et al.<sup>16</sup>, examined the effects of four different types of exercise modalities over the eight-week period in the aerobically trained males; two of the programs were of the aerobic type, continuous, and of long duration (i.e., 25–45 min between 70% and 85% HR<sub>max</sub>) while the other two were the high-intensity intervals (i.e., 47 repetitions of 15-sec activity at 90%–95% HR<sub>max</sub>, and 15 sec recovery at 70%, vs. four 4-minute 90%–94% and 3-minute recovery at 70% HR<sub>max</sub>)<sup>16</sup>. Upon the completion of eight weeks, only those who participated in the high-intensity groups reported the significant increases in VO<sub>2max</sub>: (pre: 60.5 ± 5.4 vs. post: 64.4 ± 4.4 mL/kg/min<sup>-1</sup>) / (pre: 55.5 ± 7.4 vs. post: 60.4 ± 7.3 mL/kg/min<sup>-1</sup>), respectfully<sup>16</sup>. Additionally, six weeks of a high-intensity cycling interval training was enough to elicit a 7 mL/kg/min<sup>-1</sup>, which was comparable to 5 mL/kg/min<sup>-1</sup> observed following the aerobic training protocol<sup>9</sup>. The findings of the improved VO<sub>2max</sub> following the HIFT intervention in the current study support the literature in that that a high-intensity based training is an effective mode for the improved VO<sub>2</sub>.

Another important consideration is the time frame of the current study. Though it is well established that the aerobic capacity improves following multiple weeks of training (i.e., ≥ eight-weeks)<sup>7,16,17</sup>, there is little empirical evidence regarding the training protocols less than eight-weeks of training? in the physically active participants. In a study by Burgomaster et al.<sup>18</sup>, the researchers observed an almost the

two times greater improvement in muscle endurance after two-weeks of the high-intensity sprint training in the physically active participants. Despite this, no improvements in the oxygen consumption was observed following the two-week intervention. Similarly, the seven-day high-intensity training (HIT) overload training study was performed on a competitive cyclist and found the significant improvements in time trial performance, despite no observed change in the physiological factors<sup>19</sup>. Conversely, the two-week sprint cycling training protocol improved peak oxygen consumption by 7% in the sedentary and overweight participants<sup>20</sup>. The two-week sprint cycling study, in conjunction with the current findings, supports the notion that the rapid improvement in aerobic adaptation can occur following HIFT. Currently, there is a limited number of studies that provide insight into the mechanisms responsible for the early aerobic adaptation following HIFT-like programming. Due to the conflicting results within the current literature, further research in this area is required.

Though it is outside the scope of the current study, several physiological factors may have played a role in the rapid improvement in the aerobic performance. A commonly observed adaptation following HIT is an increase in peripheral vascular structure and function. This results in the improved delivery of O<sub>2</sub> to the tissues and subsequently improved the a-vO<sub>2</sub> difference<sup>21</sup>. However, it is unlikely that the vascular structure increased during the four-weeks of HIFT; rather, it is more probable that an improvement of vascular function and control contributed to the observed improvement in VO<sub>2max</sub>. An important distinction to make between HIT and HIFT is the modality of the exercise being used. The most of the aerobic and HIT training protocols use a single modality such as running or cycling<sup>9,20,22</sup>, while the HIFT training incorporates aerobic, anaerobic and resistance-based exercise all in one workout. Many of the exercise movements in HIFT include upper body movements (e.g., push-press, push-up, pull-up, etc.). The reason this is important to note is the presence of the pressor reflex, which has been shown to more rapidly elevate the HR and place more stress on hemodynamics<sup>8</sup>, thus presenting a potential mechanism for adaptation.

#### *Anaerobic markers*

Each participants in the study performed the Wingate Test before and after the four-week HIFT intervention. The significant increases in the post-peak and post-mean power output were observed both in males and females. These results support the hypothesis that the improvements in the anaerobic performance would occur following the short four-week intervention. It is well-established that the participation in the HIT-like programming results in the anaerobic im-

provements (i.e., peak power and mean power)<sup>15,23</sup>. For instance, the untrained individuals showed improvements in the anaerobic performance (5%–28%) following the HIT training ranging from 2 to 14 weeks<sup>23</sup>. Conversely, a two-week sprint interval training program in the physically active participants improved the anaerobic performance, which was suggested to happen because of the improved cellular buffering systems<sup>22</sup>. This supports the findings of the current study, which observed an increase of the mean power output 3.5% (male) and 5.5% (female) and peak power output 6.4% (male) and 14.1% (female).

Though the participants of this study were physically active, they were not regularly engaged in the high-intensity exercise, and thus, they were more liable to respond to the HIFT training through the principle of the anaerobic overload. Therefore, it is possible that these “physically active subjects” behaved similar to the untrained person regarding the anaerobic training. The general postulation regarding adaptation is related to the improvements in the muscle buffering capacity<sup>23</sup>, improved the muscle quality<sup>21</sup> and glycogen stores<sup>21,22</sup>.

#### *Study Limitations*

Though this study was a novel attempt to examine the influences of a four-week HIFT intervention of physiological markers of fitness in physically active individuals, it was not without its limitations. The sample size was a limiting factor of this study, though the overall effects were great enough to observe significant changes, a larger data set would provide better insight into the influences of this intervention. Furthermore, future studies should include multiple time points that span a longer period of time. The current study provides a needed understanding of the early influences of a HIFT intervention; however, a longer timeline would allow for a better understanding of the rate and degree of physiological adaptation. Lastly, the biomarkers of health such as lipid profile and glucose regulation should be examined pre and post HIFT intervention to provide a more holistic approach to examining adaptation.

#### **Conclusion**

HIFT demonstrated the rapid physiological improvements in muscular strength, aerobic capacity and anaerobic capacity, following the four-week intervention in the physically active participants. Given the starting training status of the participants and the relatively short duration of the intervention, these findings demonstrate HIFT to be a unique and an effective method of training to induce the overall markers of fitness.

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## Discrepancies between clinical and autopsy diagnosis of cause of death among psychiatric patients who died due to natural causes. A retrospective autopsy study

Neslaganje između kliničkih i autopsijskih dijagnoza uzroka smrti među psihijatrijskim bolesnicima umrlim zbog prirodnih uzroka

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### Abstract

**Background/Aim.** Autopsy studies rarely investigate the causes of natural death in psychiatric population. The aim of this study was to examine the causes of death among the subjects with various psychiatric disorders in whom a clinical (pathoanatomical) autopsy was requested. **Methods.** The study group included 118 patients (65% men, 35% women, mean age  $58.2 \pm 13.6$  years) with a psychiatric diagnosis, in whom a clinical autopsy was performed. We compared the distribution of causes of natural death among psychiatric patients and other patients, representatives of the general population who died of natural causes. We also analyzed the difference between clinical diagnoses of cause of death and the autopsy findings in psychiatric patients. **Results.** Psychiatric patients died earlier than the control group (58 vs. 69 years), usually due to the respiratory (46%) and cardiovascular diseases (37%). The most common diagnoses in psychiatric patients were organic psychoses and dementias (F00-F09) and schizophrenia and schizoaffective disorders (F20-F29). Majority of the patients (55%) died in general hospitals vs. specialized psychiatric hospitals (45%) due to somatic diseases. There was a significant difference in the distribution of causes of death compared to the control group in which the cardiovascular diseases dominated. Even in 64% of psychiatric patients there was a discrepancy between the clinical diagnosis of the cause of death and definite autopsy findings. **Conclusion.** The assessment of somatic diseases in psychiatric patients is insufficient, especially in specialized psychiatric hospitals. That leads to a significant discrepancy between clinical diagnosis of the cause of death and autopsy findings. Therefore, it is necessary to pay additional attention in diagnostics and treatment of somatic diseases in these patients to improve their health care.

### Key words:

autopsy; cause of death; death, sudden; diagnosis; hospitals, general; hospitals, psychiatric; mental disorders; patients.

### Apstrakt

**Uvod/Cilj.** Obdukcijske studije se retko bave ispitivanjem prirodnih uzroka smrti kod psihijatrijskih bolesnika. Cilj istraživanja bio je ispitivanje uzroka smrti bolesnika sa različitim psihijatrijskim oboljenjima, u slučajevima gde je tražena i urađena patoanatomska (klinička) obdukcija. **Metode.** U istraživanje je bilo uključeno 118 bolesnika (65% muškaraca, 35% žena, prosečne starosti  $58,2 \pm 13,6$  godina) sa dijagnozom psihijatrijske bolesti kojima je rađena patoanatomska (klinička) obdukcija. Poređena je distribucija uzroka prirodne smrti psihijatrijskih bolesnika sa drugim bolesnicima (koji reprezentuju opštu populaciju) umrlim zbog prirodnih uzroka smrti, a ispitivane su i razlike između kliničkih i autopsijskih dijagnoza uzroka smrti kod psihijatrijskih bolesnika. **Rezultati.** Psihijatrijski bolesnici umirali su ranije u odnosu na opštu populaciju (58 vs. 69 godina), najčešće zbog respiratornih (46%) i kardiovaskularnih bolesti (37%). Najčešće dijagnoze kod psihijatrijskih bolesnika bile su organske psihoze i demencije (F00-F09) i shizofrenija i shizoafektivni poremećaji (F20-F29). Većina njih (55%) umirala je u opštim bolnicama, a 45% u psihijatrijskim bolnicama zbog somatskih oboljenja. Ustanovljena je značajna razlika u distribuciji uzroka smrti u odnosu na kontrolnu grupu u kojoj su dominirale kardiovaskularne bolesti. Kod 64% psihijatrijskih bolesnika postojale su značajne razlike u kliničkoj dijagnozi uzroka smrti u odnosu na konačan obdukcioni nalaz. **Zaključak.** Dijagnostika somatskih bolesti kod psihijatrijskih bolesnika je nedovoljna, pogotovo u psihijatrijskim bolnicama. Ovo dovodi do značajnog neslaganja između kliničkih i autopsijskih dijagnoza uzroka smrti u ovoj populaciji. Potrebno je posvetiti dodatnu pažnju ispitivanju i lečenju somatskih bolesti kod ovih bolesnika da bi se poboljšala njihova zdravstvena zaštita.

### Ključne reči:

autopsija; smrt, uzrok; smrt, iznenadna; dijagnoza; bolnice, opšte; bolnice, psihijatrijske; psihički poremećaji; bolesnici.

## Introduction

Many studies suggested that psychiatric patients have an increased risk of premature death, including both natural and unnatural causes<sup>1-8</sup>. The hypotheses about why people with psychiatric disorders are more likely to die than the rest of the population are many, different and often supported by contradictory data. It is known that psychiatric patients are more prone to death from violent causes, especially suicide or accident death<sup>3,5</sup>. On the other hand, although a correlation between organic diseases and mental disorders is evident, there is no adequate explanation of the excess morbidity and mortality of the people with severe mental illnesses (including schizophrenia, schizoaffective disorder, bipolar disorder, and depressive psychosis)<sup>5,6</sup>. There seems to be several groups of reasons for this phenomenon. The first one includes socioeconomic reasons – people with serious mental illnesses live with health disparities, which, among other things, result from social dysfunction, stigma, and direct consequences of psychopathology<sup>5,6</sup>. The second possible explanation could be the unhealthy lifestyle of people with psychiatric disorders (smoking, alcohol abuse, dietary habits, illicit drug use, physical inactivity, etc.), especially those with severe mental illnesses<sup>5-8</sup>. Beside these, there is another potential health disparity which comes from the deleterious physical consequences of a long-term antipsychotic use and their relation to cardiovascular deaths<sup>1,3,6</sup>. In the diseases such as schizophrenia, the autonomic irregularities could also provide a mechanism that could produce sudden death<sup>5</sup>. Additional reasons could be less adequate medical assistance for psychiatric patients than for people without psychiatric disorders<sup>9,10</sup>, and, furthermore, the limited ability of people with mental disorders to recognize and communicate their symptoms of organic diseases could be another possible explanation<sup>5</sup>.

Most of these conclusions came out of the large cohort studies based on registers of psychiatric patients. However, there is a small number of the autopsy studies that research natural causes of death in psychiatric patients, and most of these studies consider sudden death<sup>3,11,12</sup>. Most of these studies indicate the cardiac death (and most commonly coronary heart disease) as the most common natural cause of death in psychiatric patients.

Autopsy, as a gold standard in diagnostics, is an important tool for testing and consequently improving clinical practice<sup>13</sup>. For a long time, there has been a great interest in comparing clinical diagnoses and autopsy findings<sup>14,15</sup>. Many diagnoses that are not detected death before can be revealed by autopsy<sup>16</sup>. Moreover, some studies found the significant discrepancies between the clinical diagnosis and autopsy findings<sup>14</sup>. Still, it is not easy to find studies on concordance between the clinical diagnosis of cause of death and autopsy findings in psychiatric patients.

The aim of this study was to examine the causes of death among the subjects with various psychiatric disorders for whom a clinical (pathoanatomical) autopsy was requested.

## Methods

This 7-year (2007–2013) retrospective autopsy study was performed at the Institute of Pathology, Faculty of Medicine, University of Belgrade, Serbia. The subjects of our study were selected from all decedents who underwent the complete clinical (pathoanatomical) autopsies at the Institute. The study participants were all the deceased with psychiatric diagnosis in their medical history, submitted with the autopsy request. The psychiatric diseases were classified according to ICD-10 into the following groups: schizophrenia and schizoaffective disorders, affective disorders (depression and bipolar disorder), neurotic and somatiform disorders, alcohol addiction, psychoorganic syndrome and dementia.

The patients with psychiatric diseases accounted for about 2.5% of the performed pathoanatomical autopsies at the Institute during a 7-year period (118 out of 5,620 autopsies). The study group consisted of 118 subjects, with significantly more males, than females, 77 (65%) vs. 41 (35%), respectively ( $\chi^2 = 10.983$ ,  $p = 0.001$ ), with the average age of  $58.2 \pm 13.6$  years (median 57 years, ranging from 25 to 88 years), and no age difference between the males and female ( $58.1$  vs.  $58.5$ , respectively  $p < 0.05$ ).

The control group ( $n = 236$ ) was randomly selected as every next two autopsies following the autopsy of a subject with a psychiatric diagnosis. This group was selected to represent the “general population”, or at least, the population of the deceased whose pathoanatomical autopsies were performed at the Institute of Pathology, which covers about 80% of clinical autopsies performed in the city of Belgrade (population about 1.7 million people). The control group showed rather balanced distribution of genders (129 males and 107 females;  $\chi^2 = 1.373$ ,  $p = 0.2431$ ), with the average age of  $69.9 \pm 11.1$  years (median 70 years, ranging from 36 to 97 years).

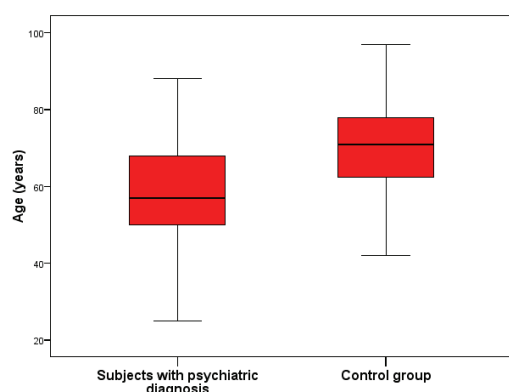
In both the study and control groups, the causes of death were classified as: malignant neoplasms, cardiovascular diseases, respiratory diseases, diseases of the gastrointestinal system, infectious diseases, or other causes.

We noted the distribution of different psychiatric diagnosis in the subjects for whom a clinical (pathoanatomical) autopsy was requested as well as the distribution of natural causes of death among these subjects compared to the control group. In addition, in the group of psychiatric patients, we also analyzed the concordance between the clinical diagnosis of their cause of death and the cause of death determined through the autopsy.

Statistical analysis was performed using the Student *t*-test for variables with the parametric distribution, and the Pearson's  $\chi^2$  test, Fisher's exact probability test and the Spearman's correlation coefficient, for variables with the nonparametric distribution. All numerical variables were tested with the Kolmogorov – Smirnov test for a normal distribution. *P* value of 0.05 was considered to be significant and *p* value of 0.01 to be highly significant. The SPSS version 17.0 (license number 106454) was used to assist the statistical analysis.

## Results

Comparing the study group with the control group showed that the psychiatric patients died significantly younger than those from the control group ( $t = -8.680$ ,  $df = 352$ ,  $p < 0.001$ ; Figure 1). There was also a borderline significance in the gender distribution with a predominance of males over females in the group of psychiatric patients compared to the control group ( $\chi^2 = 3.628$ ,  $p = 0.057$ ).



**Fig. 1 – The box and whisker plots represent the age of the group of psychiatric patients and the control group. The lower boundary of the box indicates the 25th percentile, the line within the box marks the median, and the upper boundary of the box indicates the 75th percentile. The error bars above and below the box indicate the 90th and the 10th percentiles, respectively.**

The distribution of psychiatric diseases in the study group is shown in Table 1. Organic psychoses and dementia, and schizophrenia and schizoaffective disorders were the most frequent psychiatric diagnoses ( $\chi^2 = 28.675$ ,  $df = 3$ ,  $p < 0.001$ ).

**Table 1**  
**The distribution of psychiatric diseases in the study group**

Psychiatric disease (code according to ICD-10)	Patients n (%)
Schizophrenia and schizoaffective disorders (F20-F29)	34 (29)
Affective disorders (F30-F39)	11 (9)
Neurotic and somatiform disorders (F40-F48)	1 (1)
Alcohol addiction (F10)	22 (19)
Organic psychoses and dementia (F00-F09)	50 (42)
Total	118 (100)

ICD – International classification of diseases.

**Different psychiatric diseases in the patients who died in psychiatric hospitals and those who died in general hospitals**

Psychiatric disease code according to ICD-10)	Patients (n)	
	psychiatric hospitals	general hospitals
Schizophrenia and schizoaffective disorders (F20-F29)	26	8
Affective disorders (F30-F39)	2	9
Neurotic and somatiform disorders (F40-F48)	0	1
Alcohol addiction (F10)	7	15
Organic psychoses and dementia (F00-F09)	18	32
Total	53	65

ICD – International classification of diseases.

Among the subjects with psychiatric disease, 53 (45%) individuals died in specialized psychiatric hospitals, while 65 (55%) died in general hospitals and were primarily treated for organic diseases. There was a significant difference in the distribution of different psychiatric diseases among the patients who died in psychiatric hospitals compared to those psychiatric patients who died in general hospitals ( $\chi^2 = 20.808$ ,  $df = 4$ ,  $p < 0.001$ ) (Table 2). The Table 2 shows that patients with schizophrenia died more frequently in psychiatric hospitals, while those with organic psychoses and alcohol addiction died more frequently in general hospitals.

The statistical analysis also showed that the younger subjects died more frequently in specialized psychiatric hospitals, while the older ones died more frequently in general hospitals (Spearman's  $\rho = 0.241$ ,  $p = 0.008$ ). Younger subjects more often had schizophrenia than other psychiatric diseases (Spearman's  $\rho = 0.439$ ,  $p < 0.001$ ). The subjects with schizophrenia died more often in psychiatric hospitals, while other psychiatric patients usually died in general hospitals (Spearman's  $\rho = 0.282$ ,  $p = 0.002$ ).

There was also a significant difference in the distribution of causes of death between the study group and the control group ( $\chi^2 = 18.848$ ,  $df = 5$ ,  $p = 0.003$ ). The most frequent causes of death in the study group were respiratory diseases, whereas cardiovascular diseases dominated in the control group (more than 50% of the patients) (Table 3).

**Table 3**  
**Distribution of causes of death in the study group and control group**

Cause of death	Study group n (%)	Control group n (%)
Malignant neoplasms	5 (4.2)	7 (3)
Cardiovascular diseases	44 (37)	123 (52)
Respiratory diseases	54 (46)	86 (36)
Digestive diseases	3 (3)	7 (3)
Infectious diseases	6 (5)	13 (6)
Miscellaneous	6 (5)	0
Total	118 (100)	236 (100)

The analysis showed that there were significantly frequent discrepancies between the clinical diagnosis and autopsy findings of the cause of death ( $\chi^2 = 9.797$ ,  $p = 0.002$ ), and these findings were different in 76 out of 118 cases (64%). There was a tendency to higher concordance between the clinical diagnosis and autopsy findings in general hospitals than in psychiatric hospitals (28 vs 14, respectively), however the significance in this case was a borderline ( $\chi^2 = 3.536$ ,  $p = 0.060$ ).

The cause of death in the psychiatric patients was not related to the specific psychiatric diagnosis (Fisher's Exact Test = 0.962).

## Discussion

In this study, the psychiatric patients who died from natural causes were more than 10 years younger comparing to the control group, 58 vs. 69 years, respectively (Figure 1). There are several studies that showed that mortality among psychiatric patients is higher than the general population<sup>1-8</sup>. Some of them measured that patients with severe mental illness had lower life expectancy for 13 to more than 30 years compared to the general population<sup>17</sup>, while another showed a loss of 8.8 life-years in comparison with psychiatric patients and the general population<sup>18</sup>. These studies suggest that the main reasons for somatic diseases in these patients can be caused by direct complications of psychiatric disorders, their unfavorable behavioral styles and side effects of pharmacological treatment<sup>2,3,5-8,19</sup>. Some of the studies found no other explainable cause of death, but the mental illness itself<sup>3</sup>. Of note, patients with psychiatric diseases often do not receive sufficient check-ups regarding their somatic conditions<sup>20,21</sup>. Although these conclusions came out mostly from the large cohort studies based on registers of psychiatric patients, they all could be generally applied to our retrospective autopsy study.

Although there were significantly more males than females in our study group, there was only a borderline significance in the male predominance comparing the study group to the control group. This means that autopsies are more commonly performed on male than female subjects in general. However, we can assume that increasing sample size would have led to statistical significance and that the male predominance among subjects with psychiatric diagnosis really exists. The explanation for the male predominance could lie in the fact that our study group also included chronic alcohol abuse which is a type of hazardous behaviour, generally more common in males than females.

Organic psychoses, followed by schizophrenia and chronic alcohol abuse were the most frequent among psychiatric diagnoses in the entire study group. On the other hand, schizophrenia was the most common mental disease in the subjects from specialized psychiatric hospitals, whereas organic psychoses, alcohol addiction and depression occurred more often in subjects from general hospitals. This implies that patients with more severe psychiatric diagnosis, such as schizophrenia, probably spent more time in specialized institutions. It could also imply that medical service is less available and probably less accessible, especially for the patients with dementia<sup>9,10</sup> and that people with mental disorders have the limited ability of to recognize the symptoms of organic diseases or react to them<sup>5</sup>, which could refer to the patients with dementia and depression, as well as chronic alcoholics.

According to our data, the younger patients were more likely to suffer from schizophrenia and die in specialized psychiatric hospitals. This could mean that these patients had

more severe psychiatric condition which manifested earlier in life. In contrast, the older psychiatric patients suffered more often from organic psychoses, alcohol addiction and depression, and they deceased in general hospitals, thus, having been treated for nonpsychiatric diseases. Similarly, other studies show that there is a lower prevalence of severe mental disorders, such as schizophrenia, mood and alcohol-induced disorders, in the late than in the early adult life<sup>22</sup> as the number of new generation of older people affected is lower than the number of prevalent cases who die early, and this is explained by the premature mortality in the affected younger individuals. Consequently, older people with severe mental disorders consist of a group of survivors and a relatively small number of new cases. Old survivors could, conceivably, have had a more benign course of illness or healthier lifestyle than those who died early, whereas new cases would have had only limited exposure to the potentially detrimental effects associated with their illness<sup>22</sup>.

One of the major results of our study was a significant and quite big discrepancy between clinical diagnosis and autopsy findings of the cause of death, practically in two thirds of the cases. This discrepancy seemed to be higher in the subjects who died in psychiatric hospitals. This could suggest that quality of general medical care in general hospitals is higher than in psychiatric hospitals, with more accurate clinical judgment of the cause of death. One of the problems lies in the fact that mental and physical health care are separated, and communication between general and psychiatric institutions is insufficient, including partial and uncoordinated approaches to somatic or mental conditions as well as weak communication between general and psychiatric institutions<sup>23</sup>. Even in rich and developed countries, psychiatric patients often have lower somatic "health care utilization" and are less likely to be thoroughly examined for somatic diseases<sup>20,24</sup>. Also, there is a constant decline in the autopsy rates and it appears to be a global phenomenon, despite the significant discrepancies between the clinical and autopsy determined cause of death<sup>25,26</sup>. The reason for this could lie in more and more common judicial proceeding due to potential medical errors and an autopsy is often the proceeding that exposes some omissions in the medical procedures, diagnosis and treatment<sup>26</sup>. Our study, again, stresses the importance of the autopsy diagnosis of cause of death especially in psychiatric patients.

Another important result of our study was the difference in causes of death in the psychiatric patients compared to the control group. Most of the studies considered cardiovascular death to be the most common natural cause of death in psychiatric patients<sup>2,3,5-8,11,12,27,28</sup>, explaining the increased risk for cardiovascular diseases by higher vulnerability, reduced access to health care services, lower adherence, biological alterations, use of psychiatric medication, and higher frequency of classic cardiovascular risk factors (smoking, obesity, diabetes mellitus, metabolic syndrome)<sup>8,19</sup>. A recent autopsy study on 51 patients with schizophrenia indicated that sudden death in those patients was in most cases due to the acute myocardial infarction<sup>29</sup>. A recent large-scale meta-analysis confirmed that the pa-

tients with severe mental illness are at a significantly higher risk of cardiovascular diseases and related mortality, which is particularly aggravated in the cases with elevated body mass index and use of antipsychotic medication<sup>30</sup>. Likewise, our study showed a high proportion of cardiovascular diseases as a cause of death; however, there was even a higher prevalence of respiratory diseases compared to cardiovascular ones. This could be, in fact, explained by the same reasons mentioned above, including smoking as a common factor for both respiratory and cardiovascular diseases.

There are several limitations of the study. One of them was that no toxicological analyses were performed since these were the pathoanatomical autopsies. Therefore, in clinical terms, there were no indications of poisoning. On the other hand, since these analyses were not performed, we cannot exclude the possibility that in some of these cases, the blood level of psychiatric or other drugs could have been above the therapeutic level and could have influenced the occurrence of death. The other limitation includes the possible underestimation of the presence of psychiatric diseases in the cases where the autopsy was performed, especially in the patients treated outside the psychiatric hospitals for organic diseases. In such cases, a psychiatric diagnosis might not be included in the history. This way, the psychiatric diagnosis

might have been overlooked, or considered insignificant by the clinicians, especially in cases with mild disorders, neurotic and somatiform disorders (only one such diagnosis was noted in our sample). Therefore, this study practically considered causes of death among the subjects with severe psychiatric illness.

### Conclusion

The presented retrospective autopsy study showed that psychiatric patients die prematurely compared to the general population, most commonly due to respiratory and cardiovascular diseases. Our study also indicated that there was probably less accurate and/or less thorough diagnostic processing of somatic diseases in psychiatric patients, especially in specialized psychiatric hospitals. That led to a significant discrepancy between the clinical diagnosis of cause of death and autopsy findings. These findings are in line with a general notion that psychiatric patients have a poor access to quality health care and emphasize that the health care system has to be modified so that it can overcome current insufficiencies in treating these patients. Finally, the autopsy is, and must remain, an important tool in assessing and consequently improving clinical practice in psychiatric patients.

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## Video head impulse test in children after cochlear implantation

### Video *head* impuls test kod dece nakon kohlearne implantacije

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#### Abstract

**Background/Aim.** Cochlear implantation (CI) is a therapeutic modality that provides a sense of sound to children and adults with profound sensorineural hearing loss or deafness. The aim of this work was to evaluate the lateral semicircular canal function using a high frequency video head impulse test in children after CI. **Methods.** A prospective descriptive study included 28 children (6–17 years old) with profound sensorineural hearing loss and unilateral CI. The control group included 20 healthy children with normal hearing. The measurement of vestibular function of the lateral semicircular canal was performed using video head impulse test. After cochlear implantation, the children underwent the vestibular testing. Values vestibulo-ocular reflex of lateral semicircular canal were measured using the video head impulse test in the children with cochlear implant and the control group. The values of vestibulo-ocular reflex were compared between the group. Also, in the children with CI values of vestibulo-ocular reflex were compared between the non-implanted ear and the ear with the embedded

CI. **Results.** All 28 children with sensorineural hearing loss underwent the placement of CI through cochleostomy at the average age of  $4.8 \pm 2.92$  years. Children with the cochlear implant had a significantly lower vestibulo-ocular reflex gain of the lateral semicircular canal measured by a high frequency video head impulse test compared to the control group of children with normal hearing (T test:  $t = 3.714$ ;  $p = 0.001$ ). However in these children there was no statistically significant difference of vestibulo-ocular reflex gain in the lateral semicircular canal measured in ears with embedded CI and non-implanted ears (T test:  $t = 0.419$ ;  $p = 0.677$ ). **Conclusion.** The values of vestibulo-ocular reflex gain in the lateral semicircular canal evaluated by the video head impulse test are significantly lower in the children with a profound sensorineural hearing loss compared to the children with normal hearing. The CI did not appear to have a negative impact on the lateral semicircular canal.

#### Key words:

cochlear implantation; head impulse test; semicircular canals; video recording; hearing loss; child; adolescent.

#### Apstrakt

**Uvod/Cilj.** Ugradnja kohlearnog implanta (KI) je terapijski modalitet koji obezbeđuje osećaj zvuka kod dece i odraslih sa dubokim senzorineuralnim gubitkom sluha ili gluvoćom. Cilj rada je bio ispitivanje funkcije lateralnog polukružnog kanala upotrebom visokofrekventnog video *head* impuls testa kod dece nakon kohlearne implantacije. **Metode.** U prospektivnu deskriptivnu studiju bilo je uključeno 28 dece (6–17 godina starosti) sa dubokim senzorineuralnim gubitkom sluha i jednostranim KI. Kontrolnu grupu je činilo dvadestoro zdrave dece sa normalnim sluhom. Ispitivanje vestibularne funkcije lateralnog polukružnog kanala je izvršeno visoko frekventnim video *head* impuls testom. Nakon kohlearne implantacije, deca su povrgnuta vestibularnom testiranjem. Vrednosti vestibulo-okularnog refleksa lateralnog polukružnog kanala merene su kod dece sa KI i

kontrolne grupe i međusobno upoređivane. Takođe, kod dece sa KI načinjeno je poređenje vrednosti vestibulo-okularnog refleksa lateralnog polukružnog kanala između neimplantiranog uva i uva sa ugrađenim KI. **Rezultati.** Kod svih 28 dece sa senzorineuralnim gubitkom sluha je načinjena kohlearna implantacija kroz kohleostomu kada su deca bila prosečne starosti  $4,8 \pm 2,92$  godine. Deca sa KI su imala signifikantno manju vrednost vestibulo-okularnog refleksa lateralnog polukružnog kanala koji je meren visoko frekventnim video *head* impuls testom u poređenju sa kontrolnom grupom dece sa urednim sluhom (T test:  $t = 3,714$ ;  $p = 0,001$ ). Nije nađena statistički značajna razlika između vrednosti vestibulo-okularnog refleksa lateralnog polukružnog kanala kod ušiju sa ugrađenim KI i neimplantiranih ušiju (T test:  $t = 0,419$ ;  $p = 0,677$ ). **Zaključak.** Vrednost vestibulo-okularnog refleksa lateralnog polukružnog kanala procenjena video *head* impuls testom je značajno niža kod

dece sa dubokim senzorneuralnim gubitkom sluha u poređenju sa decom urednog sluha. Kohlearna implantacija nema negativan uticaj na lateralni polukružni kanal.

**Ključne reči:**

**kohlea, implantacija; head impuls test; polukružni kanali; video snimanje; sluh, gubitak; deca; adolescenti.**

## Introduction

A cochlear implantation (CI) is a therapeutic modality that provides a sense of sound to children and adults with profound sensorineural hearing loss or deafness<sup>1,2</sup>. The CI ensures and improves speech development, language perception, cognitive functions, providing children with the ability to develop and understand spoken language. The children with a profound sensorineural hearing loss often have dysfunctions in the vestibular nerve system which plays an important role in the development of motor functions<sup>3</sup>. There are more studies that investigated the postcochlear implantation vestibular function in adults than in children<sup>4</sup>. The testing of the vestibular function in children after CI is much more difficult than in adults due to a low level of cooperation, lack of reference data, and preoperative vestibular damage associated with sensorineural hearing loss<sup>5</sup>. The CI itself may cause lesions of the vestibular system. The main pathogenetic hypothesis for vestibular dysfunction is the damage of the vestibular receptors during surgical insertion of the electrodes in the cochlea<sup>6</sup>. The video head impulse test (vHIT) is a new tool directed to the evaluation of the semicircular and vertical canals, by a high frequency stimulation, suitable for the pediatric population<sup>7</sup>. The vHIT also provides important information about the function of the semicircular canal by the assessment of vestibulo-ocular reflexes (VOR). The gain, the vHIT output, is calculated by comparing eye and head velocity during the fast horizontal head movements in each of the six semicircular canal planes<sup>8</sup>. The children without vestibular symptoms show an average VOR gain from 0.8 to 1.02<sup>9</sup>.

The aim of this study was to analyze the function of the lateral semicircular canal of the non-implanted ear by the high vHIT in the children after the CI and to compare the results with the control group of children with normal hearing. The second aim of the study was to define whether cochlear implants affected reduction of the VOR gain of the lateral semicircular canal (LSC), ie., compare implanted and non-implanted ears in the children after the CI.

## Methods

Sensorineural hearing loss occurs when there is a damage to the cochlea, or to the nerve pathways from the inner ear to the brain. In patients with profound hearing loss threshold is equal or worse than 91 decibels hearing level (dBHL)<sup>10</sup>. The present study included 28 children with a profound sensorineural hearing loss. All children underwent the CI. The study was performed in accordance with the ethical guidelines of the Declaration of Helsinki 1975. Specifically, the study was approved by the Institution Review Board (Faculty of Medicine, University of Novi Sad,

Serbia). The parents of children involved in the study signed informed consent form prior to participation in the study.

The inclusion criteria were as follows: children with a profound sensorineural hearing loss under the age of 18 years, unilateral CI, normal temporal bone (normal morpho-functional inner ear) and computed tomography (CT) evaluation. The children with syndromic pathology were not included in the study.

A prospective clinical study was performed at the Clinical Center of Vojvodina, Novi Sad, Serbia, the tertiary health institution and the main hospital of Vojvodina region. All 28 children underwent the CI surgery performed by one of two oto-surgeons; a standard posterior tympanotomy approach was used and electrode insertion was performed through the cochleostomy made anteroinferior to the round window. Each implanted electrode was placed completely, without resistance or complications. Auditory nerve response telemetry was obtained for each child. All embedded implants were Nucleus Freedom cochlear implant speech processors. The cause of hearing loss in all the patients was unknown. The assessment of the vestibular function was made by a battery of the vestibular tests: spontaneous nystagmus test, dynamo-static and orthostatic tests (Romberg, Unterberger) and Dix-Hallpike maneuver for benign paroxysmal positional vertigo. The evaluation of the LSC VOR was done using the high frequency vHIT.

The exclusion criteria were: age over 18 years, children with a profound sensorineural hearing loss and syndromic pathology and the children with the bilateral CI.

The control group included 20 children with normal hearing.

### *Video head impulse test procedure*

The vHIT is a test for assessing the function of LSC, described by Ulmer and Chays in 2005<sup>11</sup>. The head impulse test is a passive test using high-frequency head movements, amplitude between 10 and 20°, peak 200°/s, used for testing the VOR of each semicircular channel. The anatomical components of the VOR are the semicircular canals in the peripheral vestibular system, vestibular and oculomotor nuclei of the brain stem and the extraocular muscles. The VOR functions to stabilize images on the retinas during head movement by producing eye movements in the direction opposite to head movement. The VOR gain is defined as the ratio of the eye movement response to the head movement stimulus<sup>12</sup>. The vHIT was measured using the ICS impulse type 1085 (GN Otometrics, Taastrup, Denmark) with a small high-speed (~250 frames/s) monocular digital infrared video camera on the goggles (weighing 60 g) recording the movements of the right eye. The eye was illuminated by a low level infrared light emitted by the light emitting diode (LED). A small sensor on the goggles measured the head move-

ments. Before starting vestibulo-ocular testing, a brief calibration was performed. The testing was done in a small, sound- and light-proof rooms, in a sitting position, where a child is looking at an eye-level *target* at a minimum distance of 1 m in front of it, and activation of the convergence system may interfere with the VOR<sup>12</sup>. The head movement velocity is measured by a sensor on the goggles, and high-speed camera was incorporated for superior eye tracking. Each head movement and eye response are simultaneously shown on the screen, so the clinician could see whether the stimulus and the response were adequate<sup>13</sup>. The horizontal vHIT stimulus consisted of the rapid, passive and unpredictable head movements in both directions (the clinician placed both hands on the head of the patients, and rotated the head abruptly and unpredictably to the right or left, 20 impulses at random to the right and 20 to the left), whereas every impulse was very short<sup>14</sup>. Some authors believe that passive impulses are much more sensitive in the identification of the VOR deficit<sup>15</sup>. The parameters evaluating the LSC VOR are the mean gain (the ratio of the eye movement response to the head movement stimulus).

#### Statistical data analysis

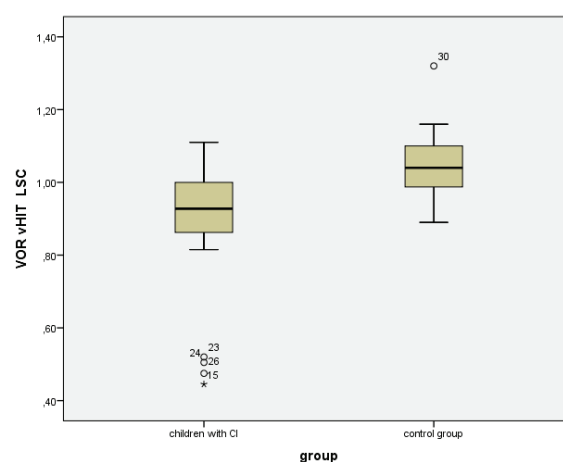
The statistical data analysis was performed using the software package Statistical Package for Social Sciences – SPSS 21. The quantitative variables were presented as the mean values (arithmetic mean) and the measures of variability (range of values, standard deviation) and qualitative variables by using frequencies and percentages. The comparison of quantitative variables between the two groups was performed using the Student's *t*-test. The differences in frequency of the qualitative variables were assessed using  $\chi^2$ -test.

#### Results

This study included a cohort of 28 children with the CI, mean age  $12.9 \pm 3.09$  years (range from 6 to 17 years). On average,

the children underwent the CI at the age of  $4.8 \pm 2.9$  years. All children were implanted unilaterally. The right ear was implanted in 18, and left in 10 children. All patients underwent the preoperative CT of the temporal bone, showing normal morphology of the inner ear, and the cause of deafness was unknown. The control group included 20 children with normal hearing, average age  $10.7 \pm 4.2$  years (from 4 to 16 years).

We found that the results of the vestibular function tests, defined as spontaneous nystagmus, oto-neurological tests, Dix Hallpike maneuver for benign paroxysmal positional vertigo, were normal in the children with CI and the control group (Table 1). There was no significant difference in the presence of vertigo in the children with the CI and the children in the control group (Table 1). However, the children with the CI had the significantly lower values of the LSC VOR gain vHIT compared to the control group:  $0.89 \pm 0.18$  vs.  $1.04 \pm 0.09$ , respectively ( $t = 3.714$ ;  $p = 0.001$ ) (Figure 1).



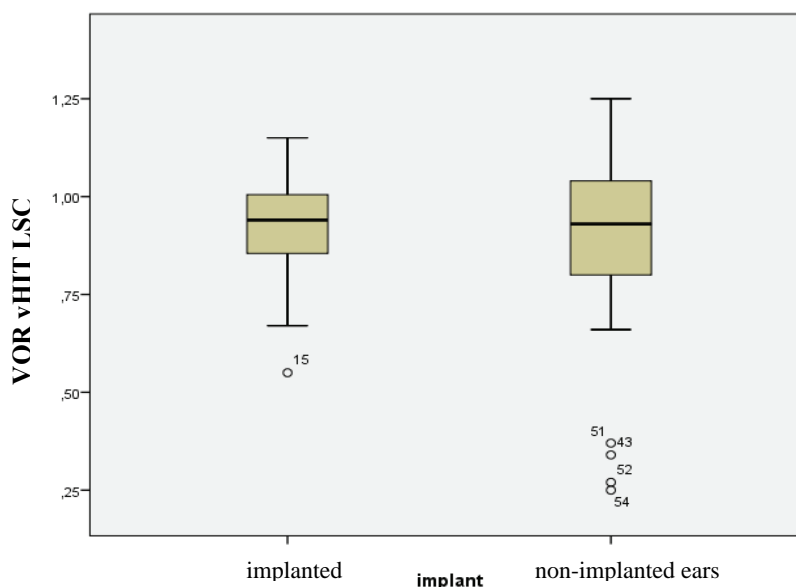
**Fig. 1 – Values of the lateral semicircular canal (LSC) vestibulo-ocular reflexes (VOR) gain using video head impulse test (vHIT) between the children with cochlear implantation (CI) and the control group.**

**Table 1**

**The investigated parameters in the group of children with the cochlear implantation (CI) in comparison to the control group**

Variables	Children with CI (n = 28)	Control group (n = 20)	<i>p</i>
Age (years), mean $\pm$ SD	$12.9 \pm 3.09$	$10.7 \pm 4.2$	0.042
Sex (male/female), n (%)	11 (39.3)/17 (60.7)	13 (65)/7 (35)	0.079
VOR gain vHIT	$0.89 \pm 0.18$	$1.04 \pm 0.09$	0.001
Vestibular function tests			
vertigo (positive/negative), n (%)	1 (3.6)/27 (96.4)	0 (0)/20 (100)	1.00
spontaneous nystagmus (positive/negative), n (%)	0 (0)/28 (100)	0 (0)/20 (100)	–
Romberg's test (positive/negative), n (%)	0 (0)/28 (100)	0 (0)/20 (100)	–
Unterberger's test (positive/negative), n (%)	0 (0)/28 (100)	0 (0)/20 (100)	–
Dix Hallpike maneuver test (positive/negative), n (%)	0 (0)/28 (100)	0 (0)/20 (100)	–
supine roll test (positive/negative), n (%)	0 (0)/28 (100)	0 (0)/20 (100)	–
deep head hanging maneuver (positive/negative), n (%)	0 (0)/28 (100)	0 (0)/20 (100)	–

**SD – standard deviation; VOR – vestibulo-ocular reflex; vHIT – video head impulse test; *p* – statistical significance.**



**Fig. 2 – Values of the lateral semicircular canals (LSC) vestibulo-ocular reflexes (VOR) gain using video head impulse test (vHIT) between implanted and non-implanted ears, in children with cochlear implantation.**

A statistically significant difference was not obtained ( $t = 0.419$ ;  $p = 0.677$ ) for the values of the VOR gain vHIT between the implanted ( $0.91 \pm 0.14$ ) and non-implanted ears ( $0.86 \pm 0.26$ ) (Figure 2).

### Discussion

The mechanisms of vestibular function damage after the CI are still unknown. However, they may be related to a number of etiological factors as well as trauma caused by electrode insertion, labyrinthitis, perilymphatic postoperative fistula, endolymphatic hydrops, intraoperative perilymph gusher, electrical stimulation of the vestibular implant, benign paroxysmal positional vertigo<sup>16–19</sup>. It has been reported that a postoperative vestibular damage after the CI occurs in 6%–80% of adult patients, and in 9%–50% of implanted children<sup>4, 20, 21</sup>. The post-mortem histological examination of the cochlea of the temporal bone, showed that the CI leads to the structural damage of the inner ear, and in particular cochlear hydrops caused by endolymphatic flow obstruction and the posterior labyrinth<sup>22</sup>. The impact of the CI on the postoperative vestibular function is difficult to assess if it was not preoperatively evaluated. The relevant literature describes the vestibular dysfunctions in 30%–70% of the children with hearing loss<sup>4</sup>. Although the CI affects the vestibular function, after the implantation, children show rapid compensation of the sensory deficit and therefore the vestibular damage may not be detected at all<sup>21</sup>. This is the reason for discrepancy between the subjective symptoms and the results of vestibular tests, which are objective.

The vestibular function tests are more difficult to perform in children than in adults, no matter if children have the CI or not. So far, only one study evaluated the vestibular function (LSC VOR gain) in children after the CI using vHIT. Our results showed that the LSC VOR gain evaluated by the vHIT of the non-implanted ears in the children with

sensorineural hearing loss was significantly lower compared to the control group. These data can be explained by the fact that in congenital bilateral sensorineural hearing loss, the vestibular dysfunction is common, and that it was present before surgery as well. Nassif et al.<sup>5</sup> published the first paper on the examination of the LSC high frequency VOR using the vHIT in children after the bilateral CI and presented the different results. They found that the LSC high frequency VOR gain in 16 children with the unilateral and bilateral CI did not differ between the non-implanted ears in children with the CI and profound sensorineural hearing loss, and the control group of children with normal hearing. Another important finding of this study is that there was no statistically significant difference in the VOR gain high frequency vHIT between the implanted and non-implanted ears in the unilaterally implanted children with profound sensorineural hearing loss. The same conclusions were reported by other authors, using the low-frequency caloric stimulation test in adults after the CI<sup>23–25</sup>. In their study on children after the CI, Nassif et al.<sup>5</sup> also found that there were no statistically significant differences of the LSC VOR gain between the implanted and non-implanted ears. Migliaccio et al.<sup>26</sup> established that the significant vestibular dysfunctions after the CI were very rare. Wolter et al.<sup>27</sup> reported different results; namely, the children with the CI showed significantly more LSC function abnormalities than the children without CI.

According to Thierry et al.<sup>21</sup>, 50% of children had normal bilateral vestibular responses after the CI, whereas the other 50% showed various kinds of anomalies: unilateral or bilateral vestibular dysfunction, 19% of children had asymmetry of vestibular function on the side of CI, while one third of them had abnormal findings in the contralateral ear. Jutila et al.<sup>28</sup> used a motorized head impulse test after the CI and found a significantly lower LSC gain only in 4 patients out of 44 adults with the CI in the early and in 2 patients in the late postoperative period; they concluded that

high-frequency loss of vestibular function or vestibular symptoms are rare, but possible after the CI. Batuescas-Caletrio et al.<sup>13</sup> reported that one third of adult patients with the CI had changes in the LSC gain. Vestibular function after CI was examined by the HIT only in 4 studies<sup>29</sup>. Basta et al.<sup>30</sup> found no loss of saccular function by the HIT after the CI. It is assumed that persistent postsurgical instability may be due to a possible co-activation of the inferior vestibular nerve by electrical stimulation.

The literature data show many controversies regarding the impact of surgical approach on the CI, through the round window, or cochleostomy, on the vestibular function<sup>31</sup>. Some authors believe that electrode insertion through the round window is less traumatic than through cochleostomy<sup>31</sup>. Although in

our study the CI was performed through cochleostomy, it showed no negative effects on the vestibular function.

## Conclusion

The evaluation of the vestibular function of lateral semicircular canal by the video head impuls test in children is non-invasive and objective method. It is hard to explain the impact of cochlear implant on the vestibular function and findings of the vestibular tests do not correlate with subjective symptoms. The results of this study imply that the cochlear implant has no effect on reduction of the LSC measured by the high frequency video head impuls test. The cochlear implant is a safe surgical procedure with a low complication rate.

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## Oral hygiene habits and prosthodontic treatment needs in younger adolescent population of Pančevo, Serbia

Oralnohigijenske navike i potrebe za protetskim zbrinjavanjem mlađih adolescenata u Pančevu, Srbija

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### Abstract

**Background/Aim.** Dental status and oral hygiene habits are poor in young population living in deprived socioeconomic conditions. The aim of this research was to ascertain oral hygiene habits in adolescents attending high schools in urban area, determine the incidence of tooth loss at the age of 15–16 years and the gender difference. **Methods.** The epidemiological cross-sectional study included 234 randomly selected high school students. The research instruments were questionnaire (focusing on socio-demographic characteristics, habits, attitudes and behavior related to general and oral health) and clinical examination (tooth loss, normative need for prosthodontics restorations and presence of restorations). **Results.** Gender-related data comparison revealed that 32.5% of girls and 8.1% of boys had at least one tooth extracted ( $p < 0.05$ ). Also, 56.2% of girls and 75.7% of boys brushed their teeth twice a day. Caries complications were identified as the indication for tooth extraction in 82.8% of participants. The data analysis confirmed the correlation between gender and tooth loss as well as treatment needs. Tooth loss was correlated with oral hygiene habits and reasons for dental visits. Treatment need was also affected by the reasons for tooth extraction and the absence of adequate prosthodontics therapy ( $p < 0.05$ ). **Conclusion.** Sociodemographic conditions significantly influenced the number of participants with extracted teeth. A prosthodontic treatment need was influenced by the reasons for tooth extraction, adolescents' knowledge about the importance of adequate treatment and previous unpleasant experience.

### Key words:

adolescent; dental care; dental health surveys; dentures; habits; oral hygiene; tooth extraction.

### Apstrakt

**Uvod/Cilj.** Oralnohigijenske navike i dentalni status su lošiji kod adolescenata koji žive u lošijim socioekonomskim uslovima. Cilj rada bio je da se utvrdi kakve su oralnohigijenske navike adolescenata koji pohađaju srednju školu u gradskoj sredini, odredi incidenca ispitanika kojima je izvađen zub u uzrastu 15–16 godina kao i postojanje razlike u polu između proučavanih grupa ispitanika. **Metode.** U epidemiološku studiju preseka bila su uključena 234 slučajno izabrana učenika srednjih škola u Pančevu. Instrumenti istraživanja su bili upitnik (9 celina, čija su se pitanja odnosila na sociodemografske karakteristike ispitanika, navike, stavove i ponašanje u vezi sa oralnim i opštim zdravljem) i klinički pregled (gubitak zuba, normativne potrebe za protetskim zbrinjavanjem i prisutnim nadoknadama. **Rezultati.** Nađena je značajna razlika u odnosu na pol; kod 32,5% devojčica i 8,1% dečaka izvađen je bar jedan zub; 56,2% devojčica i 75,7% dečaka peru zube dva puta dnevno; 82,8% ispitanika navelo je komplikacije nelečenog karijesa kao razlog za vađenje zuba. Statističkom obradom rezultata potvrđeno je postojanje korelacije između polova i gubitka zuba kao i potreba za zbrinjavanjem. Gubitak zuba je povezan sa oralnohigijenskim navikama, ali i razlozima zbog kojih ispitanici odlaze kod stomatologa. Na potrebu za protetskom nadoknadom uticali su razlozi vađenja zuba i odsustvo adekvatne protetske terapije. **Zaključak.** Sociodemografski faktori značajno utiču na procenat ispitanika kojima su izvađeni zubi. Potreba za terapijom je uslovljena razlozima vađenja zuba, znanjem ispitanika o važnosti tih razloga, ali i prethodnim neprijatnim iskustvom kod stomatologa.

### Ključne reči:

adolescenti; zubi, nega i lečenje; zubi, ispitivanje stanja; zubna proteza; navike; usta, higijena; zub, ekstrakcija.

## Introduction

Oral hygiene habits are formed and learnt during the earliest period of child's life and developed and strengthened during the period of adolescence. Parents and the closest surrounding have an important role in the formation of positive health habits in children. Parents' influence is closely related to the existence and persistence of oral hygiene habits, i.e., a positive correlation has been established between socioeconomic status of the family and regular oral health maintenance<sup>1</sup>.

For the adolescents, the main sources of information related to oral health are family and school<sup>2</sup>. The dental status of the parents may define their approach towards oral health and hygiene habits, so their subjective norms determine how much attention parents devote to the formation of positive habits in their children<sup>3</sup>. Parents' knowledge about the hygiene habits is often reflected in their children's oral and dental status<sup>4</sup>. Mother's education was identified as a protective factor for the oral hygiene habits of the adolescents<sup>5</sup>. Fontanini et al.<sup>6</sup> relate the absence of the positive hygiene habits and poor use of oral health care in adolescents to the influence of family, friends and access to material goods. Better socioeconomic conditions in which a young person lives, social support and youth culture affect knowledge and attitude towards oral health, its importance and oral status and hygiene habits. Adolescents who live in better conditions, do better at school and have a large groups of friends, also have better dental status and oral hygiene habits<sup>7</sup>.

Oral health care in Serbia is freely accessible for the sensitive groups including adolescents. According to the data published by the Statistical Office of the Republic of Serbia, 9.2% of Serbian population lives below the poverty line. Dental status and oral hygiene habits are worse in adolescents living in the inadequate socioeconomic conditions. This is particularly related to rural areas, large families and families with a low level of parents' education<sup>8</sup>. The aims of this study were to determine oral hygiene habits in the adolescent population, the frequency and the main reason for dental visits, the effect of oral hygiene habits on tooth loss and normative treatment needs as well as the percentage of adolescents aged 15–16 years with at least one extracted tooth and the percentage of subjects in need of a prosthodontic rehabilitation, the effect of socioeconomic status of parents (education, employment, income) on tooth loss and the need of prosthodontic rehabilitation in adolescents.

## Methods

This epidemiological cross-sectional study included 234 first and second grade high school students in Pančevo, in the period of May–June 2015. The participants were randomly chosen. Only pupils enrolled in public schools were included. All eligible pupils (325) were invited to take part in the study, of which 234 were finally included (response rate was 72%). Their parents gave written consent and the Ethics Committee of the Faculty of Dentistry, Pančevo, approved the study. All questionnaires without information regarding gender, age and dental habits were excluded from the study.

## Research instrument

The research instrument used in this study was the questionnaire. A pilot study was conducted on 20 subjects of the same age. The aims of this pilot study were to verify the questionnaire, evaluate its clarity, introduce changes and simplified formulations if necessary.

The questionnaire comprised 9 thematic parts focusing on sociodemographic characteristics, habits, attitudes and behavior related to general and oral health as well as subjects' self-evaluation regarding confidence and successfulness. Special attention was given to the questions related to the sociodemographic characteristics of each subject and their oral hygiene habits. The questionnaire was designed by the examiners, except Oral health impact on daily performance (OIDP) that was completely included without alteration.

## Dental status examination

The dental status was determined during the clinical examination performed by two calibrated researchers. Prior to the clinical examination, the researchers were trained for consistency of data and uniform assistance to the participants to fill in the questionnaire. The clinical examination was performed in classrooms at daylight, with the participants sitting in school chairs by the window. A dental mirror and probe were used to perform the clinical examination<sup>9</sup>. The following information was gathered: the loss of permanent teeth, the need for prosthodontic restoration, the presence of prosthodontic restorations, dentoalveolar malocclusions, the need for orthodontic treatment and the presence of orthodontic appliances.

## Statistical analysis

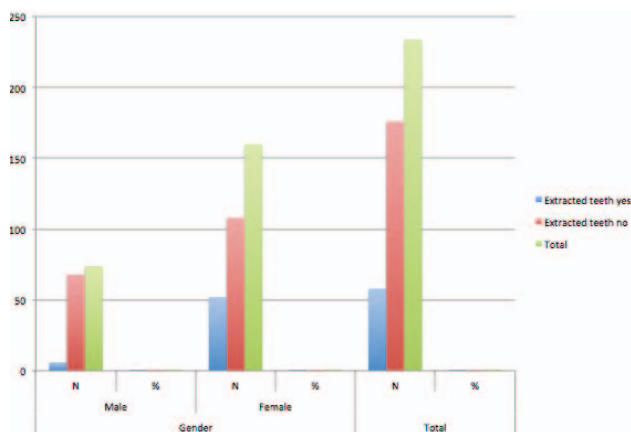
Statistical analysis was performed using the software package SPSS Statistics (version 20.0). The Pearson's  $\chi^2$  test was used in the form of a test of goodness of fit and contingency table. It was tested whether or not differences existed between the frequencies of nonparametric data for one or two parameters. The signed-rank test was used as another nonparametric statistical test. Nonparametric correlation was used to analyze the relationship between the examined characteristics in relation to data distribution.

## Results

There were 234 participants in this study, 74 (31.6%) males and 160 (68.4%) females. Even though the study was conducted in the urban area, more than a half of participants lived in the rural area (57.4%). More than two thirds of participants lived in families where parents had secondary school education (72.9% for mothers and 69.2% for fathers). Similarly, 69.5% of participants stated that their parents were unemployed (although more than two thirds claimed that their family income was above Serbian average).

The gender-related data comparison revealed a significant difference as 32.5% of girls had at least one permanent

tooth extracted compared to 8.1% of boys (Figure 1). Similar results were obtained for the second parameter, as almost one third of girls had normative prosthodontic treatment need (Figure 2). A great orthodontic treatment need was found related to dento-alveolar malocclusions (67.6% of boys and 68.8% of girls) whereas few participants were already involved in the orthodontic treatment (less than 4% in total).



**Fig. 1 – Number of participants with extracted teeth according to gender.**

The data related to oral hygiene habits revealed the following: less than 1% of participants did not brush their teeth at all, 56.2% of girls and 75.7% of boys brushed their teeth twice a day whereas nearly half of the girls brushed them three times a day compared to one fifth of the boys (43.8% vs. 21.6%). The results suggest that more than a half of the surveyed adolescents used accessory hygiene agents (65%), mouthwashes being the most frequently used (55.1%). Although the girls had worse results regarding tooth loss and normative treatment needs, the results of this study indicate that the girls (78.8%) more often than the boys (51.4%) visit dentists.

A majority of participants identified caries complications as the indication for tooth extraction (82.8%) irrespective of gender. When asked why they were not treated adequately following a tooth extraction, most participants of both gen-

ders (69% girls and 50% boys) responded that they were not informed about the importance of prosthodontic treatment. The rest of the boys indicated the lack of financial means compared to 10.3% of girls. The lowest percentage of participants stated that they did not consider prosthodontic treatment important for normal function (6.5% in total). Similar percentage of participants of both genders reported check-up or pain as the main reasons to visit dentists (42.7% and 44.4%) while only 10.3% of participants considered the tooth appearance as the reason for such visits.

Further data analysis using nonparametric correlation confirmed the correlation between gender and tooth loss as well as treatment needs (Table 1). Beside that, a significant relationship was found between the tooth loss and the parents employment status. Treatment needs were associated with family income (Table 2).

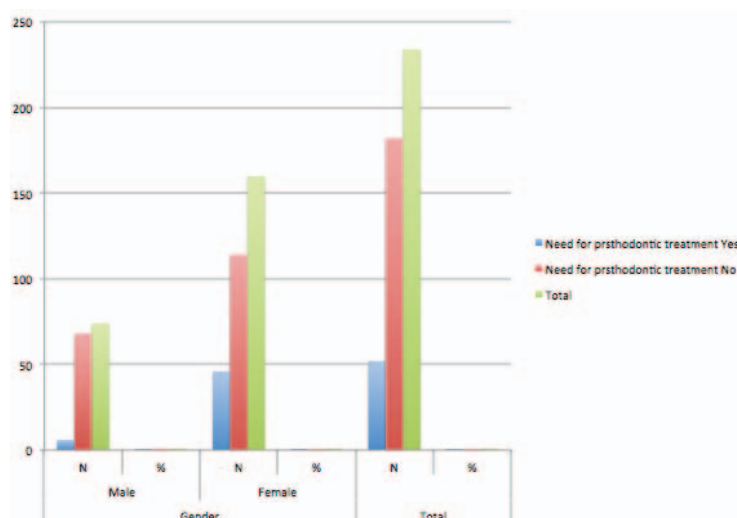
**Table 1**  
**Nonparametric correlation between gender, tooth loss and normative prosthodontic treatment need**

Variable		Gender
Extracted teeth	Ro	-0.263
	<i>p</i>	<b>0.000</b>
Prosthodontic treatment need	Ro	-0.231
	<i>p</i>	<b>0.000</b>
Presence of prosthodontic restorations	Ro	-0.063
	<i>p</i>	0.336
Presence of orthodontic anomalies	Ro	-0.012
	<i>p</i>	0.857
Presence of orthodontic appliances	Ro	-0.128
	<i>p</i>	<b>0.051</b>

**Number of participants = 234.**

**Ro – coefficient of correlation.**

The tooth loss was correlated with the oral hygiene habits and reasons for dental visits. Unpleasant experience during previous dental treatment(s) significantly affected prosthodontic treatment need. Furthermore, treatment need was also affected by reasons for tooth extraction and the absence of adequate prosthodontic therapy ( $p < 0.05$ ) (Table 3).



**Fig. 2 – Number of participants with need for prosthodontic treatment according to gender.**

**Table 2**  
**Nonparametric correlation between sociodemographic factors, tooth loss and treatment need**

Variable	Living environment	Mother's education	Father's education	Parents' employment	Family income	Lives with:	Lives in:	Family vacation	Number of family members	Do they own a computer	Do they own a car
Extracted teeth	Ro 0.091	0.021	-0.074	0.122	0.070	-0.087	-0.180	-0.099	0.002	-0.047	-0.202
	<i>p</i> 0.163	0.751	0.257	<b>0.062</b>	0.286	0.182	<b>0.006</b>	0.131	0.978	0.472	<b>.002</b>
	<i>n</i> 236	236	234	236	236	236	236	236	236	236	236
Prosthodontic treatment need	Ro 0.042	0.092	-0.009	-0.003	0.142	0.015	-0.132	-0.042	0.013	-0.063	-0.204
	<i>p</i> 0.520	0.157	0.886	0.963	<b>0.030</b>	0.814	<b>0.043</b>	0.520	0.838	0.334	<b>.002</b>
	<i>n</i> 236	236	234	236	236	236	236	236	236	236	236
Presence of prosthodontic restorations	Ro -0.108	0.017	0.034	-0.140	-0.187	0.051	0.028	-0.066	-0.017	0.021	.059
	<i>p</i> 0.098	0.790	0.605	<b>0.032</b>	<b>0.004</b>	0.432	0.667	0.313	0.791	0.744	.368
	<i>n</i> 236	236	234	236	236	236	236	236	236	236	236
Presence of orthodontic anomalies	Ro -0.062	-0.007	0.138	-0.249	-0.073	-0.125	-0.140	0.313	-0.075	0.010	-.107
	<i>p</i> 0.343	0.920	<b>0.035</b>	<b>0.000</b>	0.266	<b>0.054</b>	<b>0.031</b>	<b>0.000</b>	0.249	0.880	.100
	<i>n</i> 236	236	234	236	236	236	236	236	236	236	236
Presence of orthodontic appliances	Ro -0.029	0.035	-0.025	-0.181	-0.085	-0.003	0.057	-0.062	-0.035	0.043	.016
	<i>p</i> 0.659	0.589	0.704	<b>0.005</b>	0.195	0.966	0.383	0.342	0.592	0.507	.810
	<i>n</i> 236	236	234	236	236	236	236	236	236	236	236

Number of participants = 234.

Ro – coefficient of correlation.

**Table 3**  
**Nonparametric correlation between oral health habits, tooth loss and treatment need**

Variable	Toothbrushing - how many times a day (n = 234)	Toothbrushing - how long (minutes) (n = 234)	The use of additional hygiene agents (n = 234)	What agents do they use (n = 156)	How frequently do they have dental check-ups (n = 234)	When was the last visit to the dentist (n = 234)	Are they afraid of dental interventions (n = 234)	Did they have unpleasant experience (n = 234)	Reason(s) for tooth extraction (n = 58)	If a tooth was extracted and no treatment followed - what was the reason (n = 62)	What is the reason to visit dentist (n = 234)
Extracted teeth	Ro	-0.014	-0.192	-0.204	-0.055	0.026	0.006	0.054	-0.086	-0.169	-0.184
	<i>p</i>	0.834	<b>0.003</b>	<b>0.011</b>	0.400	0.692	0.927	0.410	0.521	0.188	<b>0.005</b>
Prosthodontic treatment need	Ro	-0.019	-0.032	-0.025	-0.086	-0.013	0.090	0.138	0.807	0.566	-0.210
	<i>p</i>	0.777	0.626	0.753	0.186	0.844	0.170	<b>0.034</b>	<b>0.000</b>	<b>0.000</b>	<b>0.001</b>
Presence of prosthodontic restorations	Ro	-0.119	0.069	-0.031	-0.038	0.060	-0.056	-0.047	0.086	-0.025	-0.054
	<i>p</i>	<b>0.068</b>	0.293	0.700	0.559	0.359	0.388	0.475	0.521	0.849	0.413
Presence of orthodontic anomalies	Ro	-0.083	-0.121	0.073	0.042	0.081	0.084	-0.067	0.225	0.310	0.029
	<i>p</i>	0.204	<b>0.064</b>	0.362	0.523	0.215	0.201	0.306	<b>0.090</b>	<b>0.014</b>	0.653
Presence of orthodontic appliances	Ro	0.045	0.041	0.055	0.173	0.122	0.096	-0.095	-0.396	-0.333	-0.014
	<i>p</i>	0.493	0.526	0.499	<b>0.008</b>	<b>0.062</b>	0.140	0.147	<b>0.002</b>	<b>0.008</b>	0.827
<b>n – number of participants; Ro coefficient of correlation.</b>											

## Discussion

During the past two decades, a major portion of industrial facilities in Pančevo, Serbia, were closed resulting in higher unemployment and lower financial status of the local population leading to changes in the population structure with the majority of families having high-school educated, unemployed parents. Irrespective of the statement related to parents' employment status, two thirds of participants stated that their family income exceeded Serbian average. The inconsistent statements may be due to the misinformation about the real family financial status or shame to state the true information. Jung et al. <sup>1</sup> recommended that surveying the adolescent population regarding socioeconomic status should be done indirectly by using sets of questions that would indicate the true financial status.

The effect of socio-demographic factors on the oral health and oral status was confirmed in a number of studies based on the Decayed, Missing, Filled Teeth (DMFT) score <sup>10–14</sup>. The same was confirmed in the present study, although the studied parameters were extracted teeth and prosthodontic treatment need. This result was expected because the lower education levels and income of parents affect primarily their attitude towards oral health and hygiene habits in the adolescents but also the ability to afford treatment not covered by insurance.

The results from this study indicated that girls had worse dental status and greater need for prosthodontic treatment, which was rather unexpected in light of the fact that girls pay more attention to health in general and oral health. Beside that, the girls are more concerned with their appearance and better informed about the importance of oral health than boys <sup>15</sup>.

The present literature contains limited information about normative treatment needs of adolescents. Some Brazilian authors reported that the percentage of participants in this age group in need of rehabilitation decreased <sup>16</sup>. Vazquez et al. <sup>17</sup> reported that 13.7% of participants in a national survey aged 15–17 years were in a need of prosthodontic treatment. The difference between their study and the present results may be explained by the fact that the whole population sample, irrespective of gender, was analyzed. The girls showed better results related to the oral hygiene habits, the use of accessory hygiene agents and the frequency of dental visits, all being in accordance with similar studies which focused on the oral hygiene habits and gender differences <sup>18, 19</sup>. Worse dental status and greater treatment needs in girls may partly be explained by the sample structure itself, with more than two thirds of participants being female.

Numerous studies focused on the hygiene habits, dental check-ups and family economic status. It was reported that the adolescents from the lowest economic strata exploited the oral health care least (even when it is free), visited dentists only in the case of an acute problem and did not have a permanent dentist <sup>10, 11</sup>. Vazquez et al. <sup>17</sup>, who reported a positive correlation between the low socioeconomic status and the number of untreated teeth, saw the same trend in a study. An average DMFT score was higher in the families with unfavorable living conditions. The authors explained this finding by

standard of living, parents' focus on existence and access to oral health care. Most authors who studied poor population groups agreed that adolescents visited dentists irregularly <sup>13, 14</sup>, went for check-ups less than once every two years and Bhola and Malborta <sup>20</sup> found that, on average, the first visit to the dentist was at the age of 12 years.

The research into oral hygiene habits showed that most participants maintained good oral health and had regular dental check-ups. These results differ from previous studies showing that the adolescents irregularly visit dentists, i.e., only when there is a problem <sup>11, 20, 21</sup>. The difference in these results may be due to the differences in the sample because the authors focused on the rural population of a lower socioeconomic status. Better results in the surveyed adolescents could be related to the effect of youth culture, access to information at school and through various media, indicating the importance of environment and social support for young people's attitude towards general and oral health.

Most participants came from the low economic class, but the effect of the sociodemographic factors on oral hygiene habits was not crucial. A great effect of the sociodemographic factors was found in categories such as tooth loss and prosthodontic treatment need. This may be explained primarily by parents' education that defines their attitude towards oral health in general but also family income where participants opt for tooth extraction as the cheapest treatment option compared to the endodontic and prosthodontic treatments. The parents' education may define adolescents' relationship to oral health and their interest, knowledge and attitudes <sup>22</sup>.

The positive hygiene habits and regular check-ups reduce the need for normative dental treatment. Despite extensive education and preventive programs, teeth are often extracted early, in childhood or adolescence. The loss of a tooth in early period of life affects the morphology of dental arches, function of masticatory apparatus as well as physical appearance, self-esteem and quality of life of a young person. The most frequent reasons for extraction of permanent teeth in the adolescent population are caries, its complications and orthodontic indications <sup>21, 23, 24</sup>. Various studies report different data on the percentage of subjects with extracted teeth in the period of adolescence, from 17.4% in Argentina, 32.3% in Turkey, 44.8% in Brazil up to 50% in a national study in Argentina <sup>16–18, 25</sup>.

Tooth loss in adolescents is a problem that may influence all aspects of life. Beside masticatory dysfunction, the esthetic appearance, self-confidence and overall quality of life of young people are affected. In the present study, 24.6% of participants had at least one permanent tooth extracted and 22% were in need of prosthodontic rehabilitation. As two thirds of the patients had dentoalveolar malocclusion, it is possible that the percentage of participants with normative treatment needs would be lower if they were included in some sort of orthodontic treatment, which would close the gaps following tooth extraction with fixed orthodontic appliances. The authors of similar studies reported different results with the percentage between 21.9% and 38.9% <sup>17, 18</sup>. The present results cannot be compared to other studies because of the

different sample structure and study design. A high percentage of participants with extracted teeth suggests that despite good hygiene habits and regular check-ups, sociodemographic factors significantly affect dental status and attitude of the adolescents towards oral health.

Only a few studies focused on the normative needs of adolescents for prosthodontic rehabilitation irrespective of the reason(s) for tooth loss both in international and national scientific literature. The available data suggest that there is a high percentage of untreated adolescents. Vazquez et al.<sup>17</sup> reported that 13.7% of adolescent subjects need some kind of prosthodontic treatment. Barbato and Peres<sup>15</sup> stated that girls were presented with more extracted teeth, which was explained in part by girls' better oral care leading to more frequent dental visits which sometimes may negatively affect the survival of teeth (overtreatment).

It is important to mention a present statistical relationship between prosthodontic treatments need and previous unpleasant dental experience, indicating that previous dental treatment itself is an important factor influencing adolescents' knowledge, attitudes and behaviour towards oral health. Previous unpleasant experience may contribute to the increased dental fear, postponing dental visits and leading consequently to tooth loss. This is corroborated with the finding that most participants had their teeth extracted due to caries complications (82.8%), which could have been prevented with adequate and timely treatment.

An important finding of this study is that the importance of an adequate treatment was not explained to the majority of adolescents with extracted teeth, which is a failure of the dental healthcare system aimed both at prevention and treatment. A lack of awareness of the consequences of tooth loss at such an early age and failure to minimize those consequences are the signs of inadequate healthcare system that should provide not only dental service but also information on the importance and ways to maintain and improve oral health.

The limitation of the present study is primarily a small number of participants, though it was representative for Pančevo. However, the survey offers a useful insight into the

dental status of an average adolescent in an urban area. It would be beneficial to enlarge this study and include a larger sample of the same age groups in various regions in Serbia. This would enable more accurate determination of the factors influencing tooth loss and treatment needs as well as their direction. The results of such a comprehensive study would be useful for planning education and preventive programs aiming at a specific age group and some specific recommendations to the dental healthcare system on how to reduce the incidence of teeth extraction at an early age and how to educate young population on the importance of timely treatment.

## Conclusion

The loss of permanent teeth in adolescence is a problem that may influence all aspects of life, because it affects the aesthetic appearance, self-confidence and overall quality of life and not just the masticatory dysfunction. In the present study every fourth participant aged 15–16 years had at least one tooth extracted and every fifth participant had some prosthodontic treatment need.

Sociodemographic conditions, such as parents' employment, family income and financial status, significantly influenced the number of participants with extracted teeth. Prosthodontic treatment need was more influenced by the reasons for a tooth extraction, adolescents' knowledge about the importance of adequate treatment as well as by previous unpleasant experience.

Girls had worse dental status and greater need for prosthodontic or orthodontic treatment despite having better hygiene habits.

Girls also had more extracted teeth and higher normative treatment needs but they visit dentists more frequently and regularly.

## Conflicts of interests

The authors have no conflict of interest to declare.

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## Defense mechanisms and quality of life in military personnel with a burnout syndrome

Mehanizmi odbrane i kvalitet života kod profesionalnih vojnih lica sa sindromom sagorevanja na radu

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### Abstract

**Background/Aim.** Professional military personnel are exposed to a number of stressors during the war as well as in peacetime conditions that can cause some hidden or manifest disorders, especially anxiety and depression, but also the development of a burnout syndrome. The aim of our investigation was to determine the defense mechanisms and subjective assessment of quality of life and anxiety in professional military personnel of the Serbian Armed Forces with the burnout syndrome. **Methods.** The cross-sectional study included a total of 55 professional military personnel, from 25 to 55 years of age, without current mental problem. In the investigation, the Maslach Burnout Inventory (MBI), Defense Style Questionnaire (DSQ-40), World Health Organization Abbreviated Instrument for Quality of Life Assessment (WHOQOL-BREF) and Beck Anxiety Inventory were used. The statistical analysis included parametric and nonparametric descriptive statistics. **Results.** Emotional exhaustion and depersonalization were present in 10.9% of subjects and in 12.7% of subjects respectively, in moderate

level, while personal accomplishment was present in 21.8% of subjects of high level. Humor was higher in the subjects with a moderate level of burnout on the personal accomplishment (PA) scale and altruism in the subjects with a low level of burnout on the depersonalization (DP) scale as well as acting-out and rationalization on the PA scale in the subjects with a moderate level of burnout. High level of anxiety was present in 14.5% of subjects. **Conclusion.** Professional military personnel with lower level of burnout were less anxious, use mature defense mechanisms and have a perception of better quality of life. When burnout is diagnosed, psychological interventions requires training through the adoption of mechanisms for overcoming everyday stress, which may affect the reduction of anxiety and the improvement of the quality of life. Professional assistance, including psychotherapy is required in severe cases.

### Key words:

burnout, professional; defense mechanisms; quality of life; military personnel; serbia; surveys and questionnaires.

### Apstrakt

**Uvod/Cilj.** Pripadnici profesionalnog vojnog sastava su izloženi velikom broju stresora u toku ratnih ali i mirnodopskih uslova koji mogu dovesti do skrivenih ili manifestnih poremećaja, prvenstveno anksioznih i depresivnih, ali i razvoja burnout sindroma. Cilj našeg istraživanja bio je utvrđivanje mehanizama odbrane i subjektivne procene kvaliteta života kod profesionalnih vojnih lica Vojske Srbije sa sindromom sagorevanja na poslu – burnout sindromom. **Metode.** U istraživanje (studija preseka) bio je uključeno 55 pripadnika Vojske Srbije starosti od 25 do 55 godina života, koji nisu imali psihičke smetnje. U istraživanju su korišteni:

Maslach upitnik kvaliteta života (MBI), Upitnik mehanizama odbrane (DSQ-40), Upitnik kvaliteta života Svetske zdravstvene organizacije (WHOQOL-BREF) i Bekov upitnik za anksioznost (BAI). Za obradu podataka je korištena parametrijska i neparametrijska statistika. **Rezultati.** Emocionalna iscrpljenost i depersonalizacija su bile prisutne u umerenom stepenu kod 10,9%, odnosno 12,7% ispitanika, dok su lična postignuća bila prisutna kod 21,8% ispitanika u visokom stepenu izraženosti. Više skorove humora pokazali su ispitanici sa umerenim stepenom burnout-a na skali ličnih postignuća (PA), a altruizma sa niskim stepenom burnout-a na skali depersonalizacije (DP), kao i acting-out i racionalizacija na skali PA kod ispitanika sa umerenim stepenom burnout-a.

*ut-a.* Anksioznost je bila prisutna kod 14.5% ispitanika u visokom stepenu izraženosti. **Zaključak.** Profesionalna vojna lica sa niskim nivoom sindroma sagorevanja na radu su manje anksiozni, koriste zrele mehanizme odbrane i imaju percepciju boljeg kvaliteta života. Kada se uoči sindrom sagorevanja na radu, psihološka intervencija zahteva edukaciju kroz učenje veština savladavanja svakodnevnog stresa, čime

se može uticati na smanjenje anksioznosti i poboljšanje kvaliteta života. U težim slučajevima je potrebna i stručna pomoć, uključujući i psihoterapiju.

#### Ključne reči:

**sagorevanje na radu, sindrom; odbrambeni mehanizmi; kvalitet života; kadar, vojni; srbija; ankete i upitnici.**

## Introduction

Professional military personnel are exposed to a number of stressors during the war but also in peacetime conditions. Those stressors can cause some hidden or manifest disorders, especially anxiety and depression, but also the development of a burnout syndrome.

A burnout in the military personnel is a construct delineating the psychological state resulting from non-effective strategies for coping with livable stress in the military environment. Long-term stress and frustration caused by effort in daily life, but also a role conflict and role ambiguity, promote or exacerbate the burnout, indicating that the military person's whole life and world are involved<sup>1-3</sup>.

In the military environment, there are some risk factors for the burnout among different work places and duties, among military flying personnel, among soldiers and officers in the zone of operational actions and among medical staff, particularly among the intensive care nurses and the nurses in the department of psychiatry<sup>4-9</sup>. It exists in people who do some jobs connected with communications with other people. Persons who aspire to perfectionism have a particular tendency to this syndrome, as those with the unreal expectations and estimations connected with themselves and their own job. Some conflicts connected with the professional roles result in emotional exhaustion and ambivalent attitude to their job, low protection of co-workers and reduce working potential and create a sense of low accomplishment and low confidence<sup>10-13</sup>.

The persons with a high burnout manifest depression and anxiety that is reflected in their quality of life. Some investigations show that there is a negative correlation between subjective assessment of quality of life and burnout. That negative correlation can have serious consequences on the health of military personnel, but also on their productivity in the workplace<sup>14,15</sup>.

Additionally, many studies have confirmed that the defense mechanisms and the quality of life are connected. It is clear that defense mechanisms constitute an important component of a person's adaptation<sup>16</sup>. The two central experiences in this regard are external stress and threat to the person's self. The focus on the anxiety and on the intrapsychic conflict is too narrow, although it is premature to dismiss the anxiety as the active component in all conditions that produce defense. The type of the coping mechanisms used in person's adaptation to the military environment that are associated with a lower burnout and psychological vulnerability is not sufficiently investigated.

In the concept of quality of life, the defense mechanisms and object relations are associated with quality of life<sup>16,17</sup>. Bell<sup>17</sup> indicates that the normal profile of object relations discloses the presence of the capability for healthy (mature) relationships while pathological object relations point out the failure of such ability. Regarding that perceptions and relationships are essential elements of the concept of quality of life, one might assume that object relations and ego defense mechanisms are associated with a person's quality of life and with the burnout syndrome, as well.

Few studies have so far dealt with the study of defense mechanisms present in the burnout syndrome<sup>18</sup>. What was proven in many studies is that the mature defense mechanisms ensure better mechanisms for coping with everyday stress by reducing the probability of significant anxiety and burnout syndrome<sup>19,20</sup>.

The aim of our investigation was to determine the defense mechanisms and subjective assessment of quality of life in the professional military personnel of the Serbian Armed Forces with the burnout syndrome.

## Methods

This cross-sectional study was conducted in the three barracks of the infantry units of the Serbian Armed Forces, in September 2016.

In this study a total of 55 randomly selected professional military personnel (officers, non-commissioned officers and contract soldiers) which were at higher professional load (duty service, guard, overtime, inability to use the days off) were included. Only those without current mental problem and who volunteered to take part in the investigation were included. Written informed consent was obtained from all participants prior to participation in the study. All participants were assured of the anonymity and that only group-level findings would be reported. The sample for the pilot study was formed with about 15% of the main study, and it made 47 respondents. After that, another 10% of respondents were added, due to the possibility if they did not fully respond to the questionnaire. In that way, it was formed a sample of 55 patients, with the decision to be in error alpha level of 0.05, a beta error of 0.1 on the border, which gave strength studies of 90%.

This study was conducted with the approval of the Ethics Committee of the Faculty of Medicine, Kragujevac. The study was approved by the General Staff of the Serbian Armed Forces, too. Confidentiality of the response was assured. A special permit for the research in the units of the Serbian Armed Forces was obtained from the Ministry of Defence.

The study was a research within the doctoral thesis that is being done at the Medical Faculty of Medical Sciences, University of Kragujevac.

### *Psychological instruments*

The sociodemographic questionnaire included the questions about the age, gender, education, marital, professional and health status.

The psychometric assessments of the burnout, defense mechanisms, quality of life and anxiety were made by using: Maslach Burnout Inventory (MBI)<sup>21</sup>, Defense Style Questionnaire (DSQ-40)<sup>22</sup>, World Health Organization Abbreviated Instrument for Quality of Life Assessment (WHOQOL-BREF)<sup>23</sup> and Beck Anxiety Inventory (BAI)<sup>24</sup>.

The MBI is the most commonly used instrument to assess burnout. The MBI consists of 22 items. According to the MBI manual, it contains three subscales, which measure 3 components of burnout: emotional exhaustion (EE), depersonalization (DP) and personal accomplishment (PA). The 9-item EE subscale assesses feelings of being emotionally overextended by one's work. The 5-item DP subscale measures having an unfeeling and impersonal response towards the recipients of one's services. The 8-item PA subscale assesses feelings of competence and successful achievement. Each item could be answered on the 7-point Likert scale ranging from "never" (= 0) to "daily" (= 6). The burnout is indicated by high scores on emotional exhaustion and depersonalization and the low scores on personal accomplishment.

The DSQ-40 is the 40-item self-report questionnaire. It is one of the most widely used psychometric instruments for assessing the ego defense mechanisms. According to the manual, it broadly categorizes 20 defense mechanisms into three hierarchies: mature, neurotic and immature defense mechanisms, similar to Vaillant's<sup>25</sup> hierarchy of the ego defense mechanisms. The defense mechanisms are classified into: (a) four mature – sublimation, humor, anticipation, and suppression; (b) four neurotic – undoing, pseudo-altruism, idealization, and reaction formation; and (c) twelve immature – projection, passive aggression, acting out, isolation, devaluation, autistic fantasy, denial, displacement, dissociation, splitting, rationalization and somatization. Each of the defense mechanism is covered by the two items, that are rated on the nine-point scale from 1 (completely disagree) to 9 (completely agree). The average scores for the two items are used to determine the individual defense mechanism<sup>22</sup>. In our investigation, we compared our results with the results of Serbian authors<sup>26</sup>.

The WHOQOL-BREF is a self-report quality of life inventory. It consists of 26 items, rated on a 5-point scale that measures the following four broad domains: physical health, psychological health, social relationships and environment. The physical health domain includes the questions about daily activities, dependence on medication and treatment, energy and exhaustion, mobility, pain and discomfort, sleep and rest and capacity to work. The psychological health domain consists of the questions about positive and negative feelings, self-esteem, body image and external image, personal beliefs and attention.

The social relationship domain consists of the questions about relationships with others, social support and sex life. The environmental domain of the scale consists of the questions about the home environment, physical security and safety, financial resources, availability of health services, leisure activities, physical environment and transportation. This shortened instrument was validated in various international field trials. It is considered appropriate to measure quality of life of the healthy population as well of ill population<sup>23</sup>. A permission to use the Serbian version is obtained from the World Health Organization (WHO).

The BAI is unspecific self-questionnaire. It served as the primary outcome for measuring the severity of anxiety in participants suffering from different primary anxiety disorders<sup>24</sup>. The BAI assesses emotional, physiological and cognitive aspects of state anxiety. It consists of 22 items, rated on the 4-point Likert<sup>27</sup> scale ranging from 0 = not at all to 3 = severely. The categorical anxiety levels consist of the minimal (0–7 points), mild (8–15), moderate (16–25) and severe (26–63) anxiety<sup>24</sup>.

### *Statistical analysis*

The statistical analysis included parametric and nonparametric descriptive statistics, depending on the nature of data. The data analysis was carried out by using the IBM SPSS (Statistical Package for the Social Sciences) software version 20.0.

For the normal distribution of all numerical parameters and scores, we used the Kolmogorov-Smirnov test. We got the results showing that in all monitored and calculated parameters and scores there was the normal distribution ( $z$  was less than 1.96, and  $p < 0.05$ ), so that it was possible to apply the parametric methods in further analysis.

The subjects, according to the values of the MBI (Emotional exhaustion, Depersonalization and Personal accomplishment) scores were divided into 3 groups, with the high, moderate and low burnout, within which the values of the defensive mechanisms, anxiety and quality of life were compared.

## **Results**

Professional military personnel were aged from 25 to 55 years (74.5% were older than 30 years). A number of the male subjects was significantly higher than the female ones (87.3% of males;  $\chi^2 = 6.047$ ;  $p < 0.01$ ). There were significantly more subjects with secondary school and university (50.9%) in relation to other categories of education completed (30.9%;  $\chi^2 = 7.449$ ;  $p < 0.01$ ). There were significantly more military personnel who were married (65.5%) compared to single ones (unmarried; 10.9%) and other (widowed/divorced; 23.6%) categories of marital status ( $\chi^2 = 7.267$ ;  $p < 0.01$ ). The presence of the somatic diseases (hypertension, diabetes) were registered in a relatively small number of subjects (7.3%) compared to those who were healthy (92.7%;  $\chi^2 = 10.119$ ;  $p < 0.01$ ). The sociodemographic variables showed that there were the statistically significant differences by all observed features (Table 1).

**Table 1**  
**Sociodemographic characteristics of military personnel (n= 55)**

Variable (listed level versus other)	% of subjects	$\chi^2$	<i>p</i>
Gender (male)	87.3	6.047	< 0.01
Age (>30 years)	74.5	5.577	< 0.01
Education (>12 years)	50.9	7.449	< 0.01
Marital status (married)	65.5	5.267	< 0.01
Health status (somatic diseases presence)	7.3	10.119	< 0.01

**Table 2**  
**Defense mechanisms, quality of life and anxiety in military personnel with burnout syndrome**

Defense mechanisms/Variable	MBI EE		MBI DP		MBI PA		BAI (anxiety)	
	low	moderate	low	moderate	low	moderate	high	minimum
	<i>p</i>		<i>p</i>		<i>p</i>		<i>p</i>	
Mature								
humor	> 0.05		< <b>0.05</b>		< <b>0.01</b>		< <b>0.05</b>	
anticipation	> 0.05		> 0.05		> 0.05		> 0.05	
suppression	> 0.05		> 0.05		> 0.05		> 0.05	
sublimation	> 0.05		> 0.05		> 0.05		> 0.05	
Neurotic								
altruism	> 0.05		> 0.05		< <b>0.05</b>		> 0.05	
reaction formation	> 0.05		> 0.05		> 0.05		> 0.05	
undoing	> 0.05		> 0.05		> 0.05		> 0.05	
idealization	> 0.05		> 0.05		> 0.05		> 0.05	
Immature								
fantasy	> 0.05		> 0.05		> 0.05		> 0.05	
projection	> 0.05		> 0.05		> 0.05		> 0.05	
dissociation	> 0.05		> 0.05		> 0.05		> 0.05	
somatization	> 0.05		> 0.05		> 0.05		> 0.05	
rationalization	> 0.05		> 0.05		< <b>0.05</b>		< <b>0.05</b>	
displacement	> 0.05		> 0.05		> 0.05		> 0.05	
isolation	> 0.05		> 0.05		> 0.05		> 0.05	
acting out	> 0.05		< <b>0.01</b>		> 0.05		> 0.05	
devaluation	> 0.05		> 0.05		< <b>0.05</b>		> 0.05	
denial	> 0.05		> 0.05		> 0.05		< <b>0.05</b>	
passive aggression	> 0.05		> 0.05		> 0.05		> 0.05	
splitting	> 0.05		> 0.05		> 0.05		> 0.05	
BAI	< <b>0.05</b>		< <b>0.01</b>		> 0.05		-	
BREF								
psychological health	> 0.05		> 0.05		> 0.05		< <b>0.01</b>	
social relationships	> 0.05		> 0.05		> 0.05		< <b>0.05</b>	
environment	> 0.05		> 0.05		> 0.05		> 0.05	
physical health	< <b>0.01</b>		< <b>0.05</b>		> 0.05		< <b>0.01</b>	

**BAI – Beck Anxiety Inventory; BREF – Quality of Life Assessment; MBI – Maslach Burnout Inventory; EE – emotional exhaustion; DP – depersonalization; PA – personal accomplishment.**

Based on the results obtained by the MBI on the EE subscale, it was observed that 49/55 (89.1%) of subjects were with the low level of burnout, 6 (10.9%) of the moderate level, but none with the high level of burnout.

On the DP subscale in 48/55 (87.3%) of subjects, it was observed the low level of burnout, in 7 (12.7%) the moderate level, but no subject with the high level of burnout.

On the PA subscale in 36/55 (65.5%) of subjects the low level of burnout was observed, in 7 (12.7%) the moderate level, and in 12 subjects (21.8%) the high level of burnout.

The DSQ-40 questionnaire showed that there were no statistically significant differences in the average score of the group of mature, immature and neurotic defense mechanisms in the subjects with the low and moderate levels of burnout.

Table 2 shows defense mechanisms, quality of life and anxiety in the military personnel with the burnout syndrome. In the

group of immature defense mechanisms, there was a statistically significant difference in the subjects with the moderate and low burnout in acting out ( $p < 0.01$ ) compared to the depersonalization scale, with the values lower in the low burnout and higher at the moderate level of burnout. There were also the statistically significant differences in rationalization and devaluation in relation to the personal accomplishment subscale ( $p < 0.05$ ), with the lowest levels of rationalization at low level of burnout, a little more higher at the high level of burnout, and the highest in the moderate burnout, while the devaluation is the lowest in the moderate burnout, a little more higher in the high burnout and the highest at the low level of burnout. The overall average values of the immature defense mechanisms were higher in those with the low burnout.

In the group of the mature defense mechanisms, humor was significantly higher in the subjects with the moderate le-

vel of burnout on the DP subscale ( $p < 0.05$ ). On the PA subscale, humor was significantly lower in the subjects with the moderate level of burnout compared to those with the low and high levels of burnout ( $p < 0.01$ ). The overall average values of the mature defense mechanisms did not differ in those with the low and moderate levels of burnout on the EE and the DP scales as well as low, moderate and high levels on the PA scale.

In the group of the neurotic defense mechanisms, there was no statistically significant difference between the average score of the neurotic defense mechanisms in people with the low and moderate levels of burnout. However, on the PA subscale altruism was higher in the subjects with the low level of burnout and the lower in those with the moderate and high levels of burnout ( $p < 0.05$ ).

The BAI questionnaire registered the low level of anxiety (0 do 21) in 46 (83.6%) of subjects, the moderate level (22 do 35) in 1 (1.8%) subject, and the high level (more than 36) in 8 (14.5%) subjects.

The BAI questionnaire score decreased with the increasing level of education of the subjects, so that the highest average values were observed in the subjects with the lowest education, and the lowest average values in those with the highest level of education.

Comparing the average scores of the results obtained on the scale of anxiety (BAI questionnaire) and burnout (MBI), it was observed that the subjects with the moderate level of burnout ( $p < 0.01$ ) had the significantly higher BAI questionnaire scores on the scale of emotional exhaustion ( $p < 0.05$ ) and depersonalization ( $p < 0.01$ ).

Comparing the average values of the results obtained on the scale of anxiety (BAI questionnaire) and DSQ 40 questionnaires, it was evident that there were the statistically significant differences in regard to humor, rationalization and denial ( $p < 0.05$ ) in the subjects with minimal and mild anxiety ( $p < 0.05$ ).

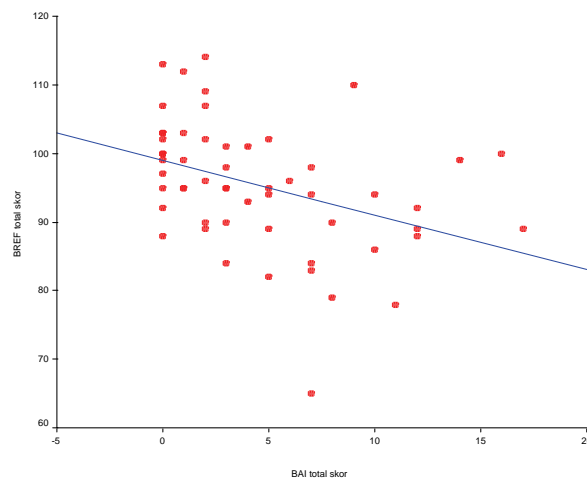
Comparing the average values of the results obtained on the scale of anxiety (BAI questionnaire) and the WHOQOL-BREF, it is observed that the subjects with the lower values of psychological and physical health ( $p < 0.01$ ) and satisfaction with social relations ( $p < 0.05$ ) had the significantly higher scores on the BAI questionnaire.

The BREF questionnaire showed that there was a statistically significant positive correlation between quality of life and the moderate level of burnout on the subscales of EE ( $p < 0.05$ ) and DP ( $p < 0.01$ ), but not on the subscale of PA.

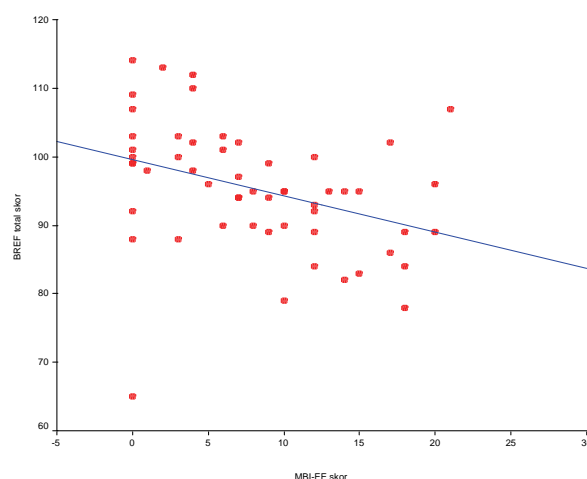
The comparison of the BREF questionnaire and MBI, DSQ-40 and BAI indicates that there was a negative correlation with all of the above scores, which means that the higher values of the BREF scores were correlated with the BAI questionnaire lower scores (Figure 1), on the EE (Figure 2) and the DP scales on the Maslach et al.<sup>21</sup> questionnaire as well as with the overall average score of the group of immature defense mechanisms on the DSQ 40, and vice versa.

There was a statistically significant correlation between the values of the BAI total score and all three subscales of the Maslach et al.<sup>21</sup> questionnaire taken individually. Thereby, there was a positive correlation with the subscales

of EE (Figure 3) and DP and the negative with the subscale of PA (Figure 4). Certainly, the higher value of the BAI score, the higher values of EE and PA subscales at the MBI were, and vice versa.



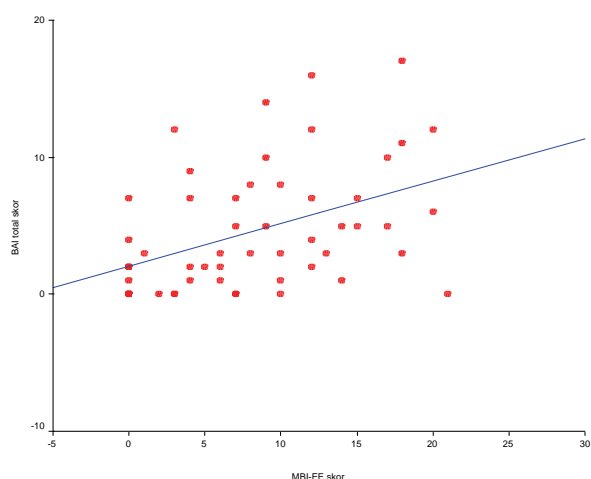
**Fig. 1 – Correlation between BREF total and BAI total.**  
BAI – Beck Anxiety Inventory; BREF – Quality of Life Assessment.



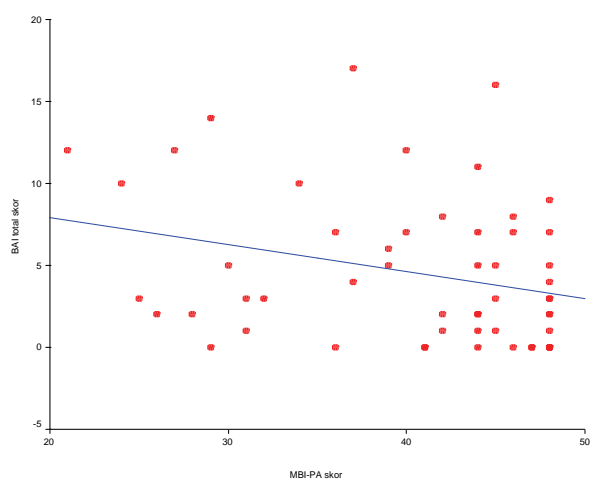
**Fig. 2 – Correlation between BREF total and MBI-EE.**  
BREF – Quality of Life Assessment; MBI – Maslach Burnout Inventory; EE – emotional exhaustion.

By analyzing the correlation of the scores obtained in the BREF, Beck et al.<sup>24</sup>, Maslach et al.<sup>21</sup> and the DSQ-40 questionnaires, it is evident that there was a statistically significant negative correlation of the BREF questionnaire score with the BAI questionnaire score, regarding the subscales of EE and DP in the MBI questionnaire and the average score of the immature defense mechanisms in the DSQ-40.

A comparison of the average values of the MBI and BAI scores showed that the subjects with the moderate level of burnout had the significantly higher scores on the scales of EE and DP, while their average values on the physical health score were lower in the subjects with the low level of burnout. Other scores were not significantly different compared to the level of burnout on the subscale of emotional exhaustion.



**Fig. 3 – Correlation between BAI total and MBI-EE.**  
BAI – Beck Anxiety Inventory; MBI – Maslach Burnout Inventory; EE – emotional exhaustion.



**Fig. 4 – Correlation between BAI total and MBI-PA.**  
BAI – Beck Anxiety Inventory; MBI – Maslach Burnout Inventory; PA – personal accomplishment.

## Discussion

Burnout is the result of prolonged stress in the workplace<sup>28</sup>. Inability to cope efficiently with workplace stressors can lead to negative psychosocial and physical outcomes. Job satisfaction level is an important factor influencing the health of military personnel. The military personnel is confronted with stress in the workplace that indicates the current, but transient, maladaptation process. Their burnout was a result of their psychological state resulting from maladaptive coping strategies with enduring stress in interpersonal communication in the military environment. It is accompanied by the physical and psychological symptoms and occurs when the adaptive capabilities of the employees are at odds with the demands of the working environment. Burnout, on the other hand, can be considered as the last stage in the process of maladaptation, when the phase of resistance gives way to collapse<sup>29</sup>.

Burnout is promoted by a role conflict and role ambiguity, or prolonged job stress and frustration caused by strain in daily life. Burnout is an indicator that the military personnel had impossible expectations of themselves in all social roles they have. They are emotionally exhausted, depersonalized, and with a low personal accomplishment. In addition, the burnout may be caused by the mismatch between the nature of the work and personality. Coping with the burnout syndrome intensifies the so-called resilience representing permanent strengths or aspects of personality that help a person to rise above life's misfortunes and build an effective style of struggle with problems, leading to the actualization of the human capacities (individual, group and community) aimed at purposeful confrontation with stressful circumstances in an emotionally and physically healthy way. Whether there will be a burnout or not, it depends on many factors along with the personality structure as a particularly significant and its reflection of practical manifestation, ranging from the powerless and weak with low frustration tolerance, to the stable and strong with high frustration tolerance<sup>28,30</sup>.

There are some investigations in the military environment where authors examined a relationship among depression, anxiety and job stress, work performance and perceptions about supervisors in military personnel. The occurrence of the burnout in the military environment was first observed in pilots and flight crew<sup>31</sup>. A survey carried out in the Wilford Hall U.S. Air Force Medical Center Mental Health Clinic showed that 60% of employees had a workplace stress, and in 52% of these it was caused by the emotional problems<sup>32</sup>. Research in the FE Warren Air Force Base (United States), showed that the military personnel in relation to the civil personnel had stress in the workplace more frequently, with more than a quarter to a significant degree, while 15% said that stress was caused by the significant emotional problems<sup>33</sup>.

Our investigation showed that more than 10% of military personnel had the moderate level of the burnout, but without the high level observed. In comparison with the investigations in other armies, the burnout in the Serbian Armed Forces was less frequently observed than in other armies.

In our investigation, a job satisfaction and job burnout appeared closely linked inversely. The military personnel showed that the job burnout is present when job satisfaction decreased because of the financial and social effects of job dissatisfaction and the damaging physical/psychological impacts of the burnout<sup>34</sup>.

Negative way of considering the stressors is associated with anxiety. The burnout syndrome was associated with anxiety, as confirmed by the results of our research, where in over 16% of the subjects, the presence of anxiety at the moderate and high level was found. Our research showed that the level of anxiety was positively correlated with the level of burnout on the EE subscale ( $p < 0.05$ ), as shown with the DP subscale ( $p < 0.01$ ). Negative reaction involves emotional focus, slow or weak reaction or absence of any attempt at solving the problem. However, despite numerous studies that have been carried out, the relationship between these two disorders is not clear. Anxiety is defined as the feeling of a floating fear, embarrassment and uneasiness. It is a normal re-

action to a stressful situation, however, if it lasts longer and if the person can not control it, then it is the anxiety disorder.

Facing the stressor, the individual activates positive or negative ways of coping with the stressful situation<sup>35</sup>. Use of the defense mechanisms alters individual perception of both internal and external reality<sup>36</sup>. In our investigation we found that in the professional military personnel the mature defense mechanisms were associated with the lower level of burnout syndrome, less anxiety and better perception of quality of life.

As shown by the results of our research, the mature defense mechanisms were associated with a good attitude to stress and good mental and physical health, while in the immature defense mechanisms, better perception of quality of life was followed by the lower level of anxiety, and lower level of burnout syndrome.

In addition to the above, many studies have confirmed that the mature defense mechanisms are associated with the higher level of quality of life<sup>37, 38</sup>. Recent Integrative models of defense and coping present a more differentiated picture with consideration to these issues: coping includes unconscious and conscious strains, defense and coping serve very similar functions, adaptiveness can be determined in quantitative (coping) and qualitative (defenses) terms and the question of stability of coping and defense needs to be more completely explored empirically. Moreover, the nature of the underlying fear can be theoretically differentiated and related to the difference between defense and coping<sup>16, 17</sup>.

People who developed the mature mechanisms are generally happier and have better mental health than individuals who use immature mechanisms. According to DSM-IV, the mature level of defensive functioning "results in optimal adaptation in the handling of stressors," whereas these defenses strive to "maximize gratification and allow the conscious awareness of ideas, feelings, and their consequences, promoting an optimum balance among conflicting motives"<sup>30, 39</sup>.

Using of altruism and humor as the "mature" defense mechanisms helps the military personnel to cope with tense or stressful situations<sup>37</sup>. In our research we obtained results that showed the greatest impact on the protection of the burnout on the subscales of depersonalization. The strongest impact on the level of anxiety had humor, while the most protective influence had humor and altruism on the scale of personal satisfaction.

Using humor, they make fun of uncomfortable situations or express unpleasant thoughts in a humorous way, allowing the military personnel to tolerate uncomfortable situations and to openly express feelings and thoughts without personal discomfort or immobilization and without producing the feeling of discomfort in others. Looking for a funny facet in military environment, in which they lack control, can help them to bear it, and it can even be an altruistic act in helping others to cope better as well.

The presence of altruism could be explained by a personal satisfaction with a successful professional functioning. The military personnel undergo a vicarious experience through constructive and instinctually gratifying service to others. Altruistic behavior, an act of goodwill to another person, can be used as a way of diffusing a potentially anxious situation. Altruism may be used as a defense mechanism, by

being especially helpful to a person who they feel might dislike them or neutralizing an argument with positivity and kind words. Appreciation therefore plays a unique role in social contacts. They feel thankful when they benefited from someone's costly, intentional, voluntary effort on their behalf<sup>40</sup>.

In addition, in our research, the immature defense mechanisms, primarily acting out, proved to be a defense mechanism that are used by more persons with the moderate levels of burnout in the DP subscale, which is in accordance with the results of numerous studies where this defense mechanism is associated with maladjustment behavior<sup>41</sup>. Rationalization and devaluation have a significant influence on the development of the burnout subscales measured by the subscale PA. Some researches show that the immature defense mechanisms are significantly more likely used in the anxiety and depressive disorders and that devaluation and projections are significantly higher in the depressive disorders compared to the anxiety disorders. Since the components of all three disorders intertwined, we can say that more investigations are needed to differentiate the defense mechanisms important for the development of burnout<sup>42</sup>.

Mutual comparison of the BREF questionnaire and the MBI, DSQ-40 and the BAI indicated that there was a negative correlation with all of the above scores. That means that the higher values on the BREF scores are correlated with the lower scores on the BAI questionnaire, on the emotional exhaustion (EE) and depersonalization (DP) scales on the Maslach et al.<sup>21</sup> questionnaire as well as with the overall average score of the group of immature defense mechanisms on the DSQ 40 and vice versa.

There was a statistically significant correlation between the values of the BAI total score and all three subscales of burnout, which is consistent with the results of other studies<sup>43, 44</sup>. Therefore, on the basis of our study, learning the adequate mechanisms for overcoming everyday stress significantly influences the reduction of the appearance of burnout and to become aware of the mechanisms that act protectively, further investigations are needed in this area.

### Limitation

Our investigation of burnout was conducted as a pilot study on a relatively small sample of the Serbian Armed Forces. Further investigation should be focused on a much larger sample size of the professional military personnel of the Serbian Armed Forces (300 military personnel), which would allow insight into the presence of burnout among the different categories of military personnel (officers, non-commissioned officers and contract soldiers). In addition to further investigations, this issue could indicate certain differences in the occurrence of burnout, depending on the type of job position, years of service, age and gender of the subjects.

### Conclusion

The professional military personnel with a lower level of burnout at work were less anxious, use mature defense mechanisms and have a perception of better quality of life.

When burnout is diagnosed, the psychological interventions requires training through the adoption of mechanisms for overcoming everyday stress, which may affect the reduc-

tion of anxiety and the improvement of the quality of life. Professional assistance, including psychotherapy is required in severe cases.

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## Hypoplasia of the ipsilateral internal jugular vein is associated with worse outcome in acute anterior circulatory stroke

Hipoplazija ipsilateralne unutrašnje jugularne vene je udružena sa lošijim ishodom akutnog moždanog udara u prednjoj cirkulaciji

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### Abstract

**Background/Aim.** Disruption of cerebral venous blood drainage leads to cerebral venous congestion, an increase in intracranial pressure and decrease of the cerebral perfusion pressure. The exact role of the cerebral venous circulation in acute stroke is not yet known. The main blood drainage from the brain and the superficial parts of the face and neck is drained by a paired internal jugular vein (IJV). Congenital anomalies of IJV may disrupt the blood collection from the brain, which leads to congestion of the cerebral venous circulation. The aim of our study was to determine the association between the hypoplastic ipsilateral IJV and clinical outcome of patients with acute ischemic anterior circulation stroke. **Methods.** This prospective case series study involved the patients with the anterior circulation stroke and ultrasonographic criteria for unilateral hypoplasia of the ipsilateral IJV. Data from the 74 consecutive patients with acute anterior circulation stroke admitted to the Special Hospital for Cerebrovascular Diseases "Sveti Sava", Belgrade, from September 2015 to January 2016 were included. Ultrasonography of IJV diameter and the collection of the

hemodynamic data were performed in all patients. Neurological deficits on admission were evaluated using the National Institutes of Health Stroke Scale (NIHSS) score. The clinical outcome was assessed using the modified Rankin Scale (mRS) score (from 0 to 6) at 30 days or at discharge, whichever occurred sooner. Good and poor outcomes were defined as an mRS score of 0–2 and 3–6, respectively. **Results.** Ipsilateral hypoplastic IJV was diagnosed in 13 (17.6%) patients with anterior circulation stroke. In this group, 9 stroke patients (69.2%) had mRS  $\geq 3$ . Of the remaining 4 patients with mRS  $\leq 2$ , three had bilateral hypoplasia of IJV and one patient had smaller diameter of the IJV, but did not fulfill the ultrasonographic criteria for hypoplastic venous anomaly. **Conclusions.** In our case, a series of the patients with anterior circulation stroke with ultrasonographic criteria for unilateral hypoplasia of the ipsilateral IJV (on stroke side) have worse clinical outcome compared with the patients with bilateral hypoplasia.

**Key words:**  
brain infarction; jugular veins; echocardiography, doppler; treatment outcome.

### Apstrakt

**Uvod/Cilj.** Poremećaj odvođenja venske krvi iz mozga dovodi do cerebralne venske kongestije, povećanja intrakranijalnog pritiska i pada cerebralnog perfuzionog pritiska. Tačna uloga cerebralnog venskog sistema u akutnom moždanom udaru još uvek nije dovoljno poznata. Glavni izvodni put krvi iz mozga, gornjeg dela lica i vrata su unutrašnje jugularne vene (VJI). Urođene anomalije VJI remete odvođenje venske krvi iz mozga, što dovodi do kongestije u venskom sistemu mozga. Cilj ove studije je bio da utvrdi povezanost između hipoplazije istostrane VJI i kliničkog ishoda akutnog ishemijskog moždanog udara (AIMU) u prednjoj cirkulaciji. **Metode.** Ova prospektivna studija slu-

čaja obuhvatila je bolesnike sa AIMU u prednjoj cirkulaciji i jednostranom hipoplazijom istostrane VJI prema ultrasonografskim kriterijumima. U studiju su slučajnim izborom bila uključena 74 bolesnika sa AIMU u prednjoj cirkulaciji koji su hospitalizovani u Specijalnoj bolnici za cerebrovaskularne bolesti „Sveti Sava“ u Beogradu, od septembra 2015 do kraja januara 2016. Kod svih bolesnika je ultrazvučnim pregledom određivan dijametar VJI. Težina neurološkog deficita na prijemu procenjena je pomoću *National Institutes of Health Stroke Scale* (NIHSS). Klinički ishod bolesti procenjen je preko modifikovanog Rankin Skora (mRS) od 0 do 6, na dan otpusta. Dobar i loš ishod su definisani preko mRS skora. Skor 0–2 je smatran dobrim, dok je 3–6 smatran lošim. **Rezultati.** Istostrana hipoplastična VJI

dijagnostikovana je kod 13 (17,6%) bolesnika sa AIMU u prednjoj cirkulaciji. U ovoj grupi devet (69,2%) bolesnika imalo je mRS  $\geq 3$ . Od preostala četiri bolesnika sa mRS  $\leq 2$ , tri su imala obostranu hipoplaziju VJI, dok je četvrti bolesnik imao užu VJI koja nije ispunjavala ultrazvučne kriterijume za hipoplaziju VJI. **Zaključak.** Bolesnici sa akutnim infarktom mozga u prednjoj cirkulaciji sa jednostranom

hipoplazijom istostrane VJI (na strani infarkta mozga) imaju lošiji ishod u odnosu na bolesnike sa obostranom hipoplazijom VJI.

#### Ključne reči:

**mozak, infarkt; vv. jugulares; ehokardiografija, dopler; lečenje, ishod.**

## Introduction

Acute ischemic stroke (AIS) can occur as a result of cerebral artery or vein occlusion. There are a lot of data about the significance of artery circulation, but, on the other hand not so many about the influence of the venous circulation. Acute ischemic stroke occurs when the cerebral blood flow (CBF) decreases under 10–12 mL/100 g tissue/min.<sup>1</sup> CBF depends on the level of cerebral perfusion pressure (CPP). CPP represents the difference between mean artery pressure and vein pressure, which explains the influence of the vein pressure on CPP.<sup>2</sup>

In supine position, the largest part of the blood drains by the internal jugular vein (IJV). This is the biggest vein in the neck, it follows the carotid artery and it unites with the subclavian vein distally to form the brachiocephalic vein (innominate vein). Further, it runs into the vena cava superior and then into the right atrium.<sup>3</sup>

Anomalies of the IJV are not so rare and can be the cause of the venous circulation insufficiency. There are different kinds of anomalies – anomalies on the venous valve, vein hypoplasia or atresia.<sup>4</sup> Hypoplasia of IJV is the cause of venous insufficiency and the increase in the intracranial blood pressure due to the reduced blood flow.<sup>5</sup> Intracranial blood pressure increase leads to the CPP decrease and consequently enlargement of a brain infarction along with worse clinical outcome.<sup>6</sup> Stable CPP allows adequate exchange of the nutrients and oxygen in the brain tissue, which is of crucial importance in an acute stroke, and it has an impact on the survival of the tissue.

The aim of our study was to determine the importance of the presence of hypoplastic ipsilateral IJV on the outcome of acute territorial stroke of the anterior circulation by ultrasonography.

## Methods

This was a case series of patients with the acute ischemic anterior circulation stroke admitted to the Special Hospital for Cerebrovascular Diseases “Sveti Sava” in Belgrade, from September 2015 to January 2016. Seventy-four consecutive patients, older than 30 years of age, with the acute anterior ischemic stroke, were enrolled in the study. The patients with thrombosis of IJV, the acute venous stroke, history of malignancy or severe infection, were excluded. The patients in our study were assessed regarding demographic data, comorbidities and vascular risk factors. A stroke severity was assessed by the National Institutes of Health Stroke Scale (NIHSS), at admission and discharge, by

board of certified neurologist.<sup>7</sup> The eligible candidates were those with the NIHSS between 4 and 25 points. The clinical outcome was assessed using the modified Rankin Scale (mRS) score (from 0 to 6) on a discharge day. Good and poor outcomes were defined as an mRS score of 0–2 and 3–6, respectively. All patients underwent the native multiphasic computed tomography [CT-General Electric (GE), Bright speed] or the magnetic resonance imaging (MRI) (MR Signa, HDx, 1.5T, GE, Milwaukee) of the brain performed on the 1.5-Tesla system at first 72 hours after the onset of a stroke. The radiological assessment was provided by an experienced neuroradiologist. For clinical classification of ischemic strokes we used The Oxfordshire Community Stroke Project criteria to assess the brain infarct size and location. All patients in our study had total anterior circulation infarct (TACI) or partial anterior circulation infarct (PACI).<sup>8</sup>

The ultrasound examinations of IJVs were performed using the MyLab 70 XVision ultrasound machine (Esaote, Genoa, Italy) by a neurologist who was blinded for the patient's neurological conditions. We used 5–7 MHz the ultrasound (US) linear probe and US settings for the examination of venous vessels.<sup>9</sup> The IJV was scanning with a minimal probe pressure, with thick US gel. The US probe was placed on the level of cricoid cartilage on either side, or 1–2 cm above IJV valves. The patients laid on their back with their head positioned at 30°. This position was the same as one when patients were lying on a hospital bed. M-mode was used for the assessment of IJV, in transversal plane (Figure 1). The patient was breathing spontaneously and after five respirator cycles we recorded a maximum vein diameter.<sup>7</sup> M-mod was used because of the variations in the diameter of veins during the respiratory cycle. The diameter of hypoplastic IJV was  $D_{max} \leq 5$  mm.<sup>4</sup> The functional stroke outcome on a discharge day was estimated using mRS.<sup>10</sup>

The Ethical Committee of the Special Hospital for Cerebrovascular Diseases “Sveti Sava” approved the study. Informed consent was obtained by all patients.

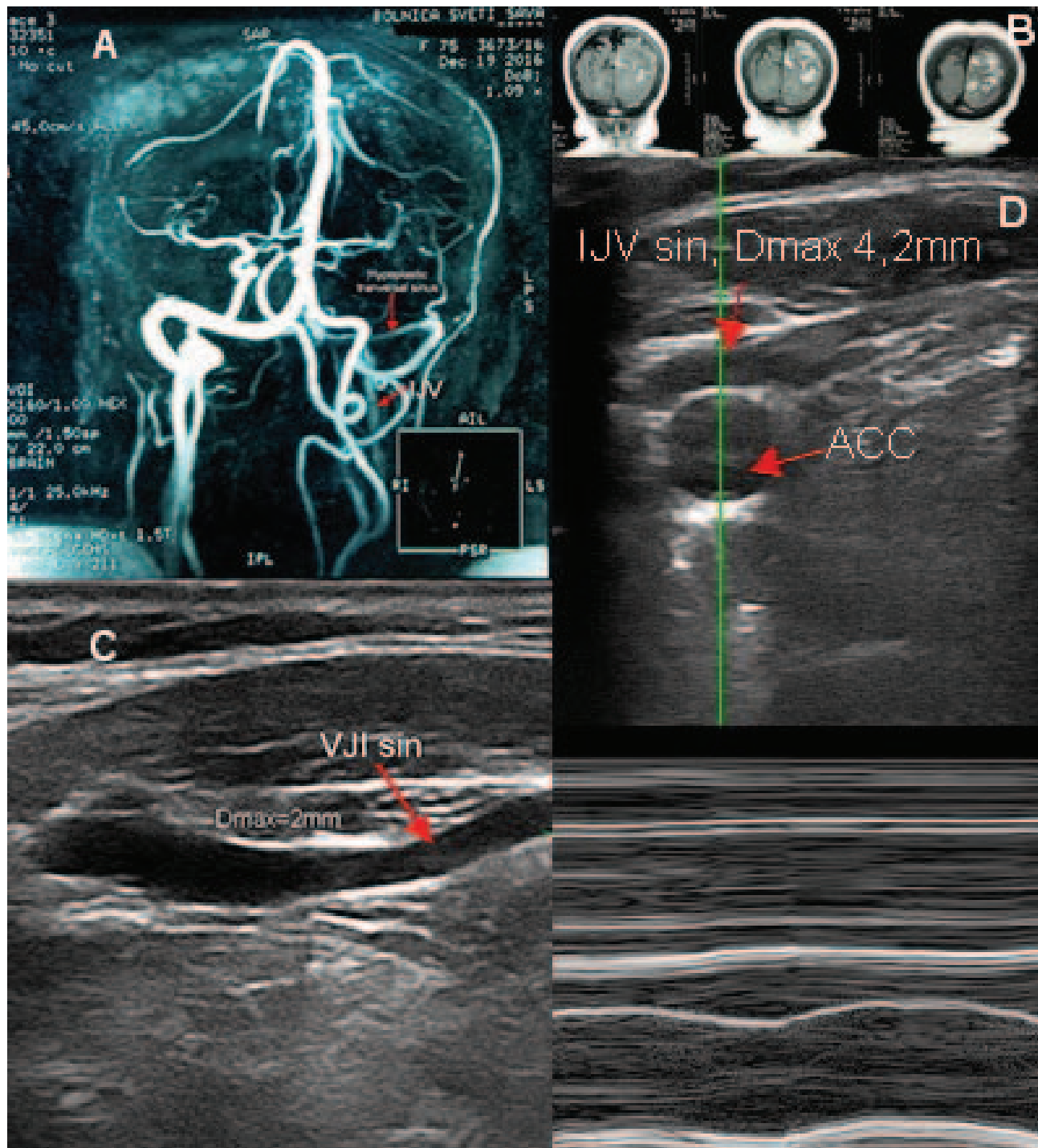
## Statistical analysis

Statistical analysis was performed using Statistical software package (SPSS). We used the methods of descriptive statistics and absolute and relative numbers, measures of central tendency and measures of dispersion. Differences in frequency distribution between the studied groups were estimated by chi-square ( $\chi^2$ ) test and confirmed by Fisher's exact test. To evaluate predictors of good and worst outcome for patients with AIS in our study we performed logistic regression analysis.  $\chi^2$  test was used to

evaluate presence of statistical significance regarding type of ischemic area and presence of IJV hypoplasia in both TACI and PACI regarding treatment outcome.

The studied variables were considered statistically significant if the probability of risk for accidental difference

between empirical and theoretical values was less than 0.05 ( $p < 0.05$ ), and highly statistically significant if the probability was less than 0.01 ( $p < 0.01$ ).



**Fig. 1 – 65-year-old woman with an acute onset of right limb weakness with neglect. A) Magnetic resonance (MR) cerebral venogram showing left transversal sinus and internal jugular vein (IJV) hypoplasia (arrows); B) Brain MR tomogram showing partial anterior circulation infarct (PACI); C) IJV on B-mode showing a maximal diameter 2mm, D. Duplex imaging IJV on M-mode at transversal plane showing small-caliber lumen of the left IJV, with a maximal diameter 4.2 mm (ACC – common carotid artery).**

## Results

This study included 74 patients, 39 (52.7%) female and 35 (47.3%) male patients. The mean age of the female study patients was 73.2 years (from 65 to 84 years of age) and of the male study patients 70.3 years (from 54 to 80 years of age). The most prevalent risk factor was hypertension (Table 1). According to the Oxford classification, of the patients with AIS, 27 (36.5%) of them had TACI and 47 (63.5%) had PACI. There was no significant difference in relation to gender – 52.1% of women had TACI and 53.5% had PACI. Right IJV was dominant in 47 (63.5%) and left IJV in 27 (36.5%) patients.

**Table 1**

**Risk factors in the patients with anterior circulation stroke (n = 74)**

Risk factor	Patients n (%)
Hypertension	62 (83.8)
Diabetes mellitus	17 (22.9)
Hyperlipidemia	11 (14.8)
Atrial fibrillation	26 (35.1)
Ischemic heart disease	22 (29.7)
Smoking	12 (16.2)
TACI	27 (36.5)

**TACI – total anterior circulation infarct.**

Sixty-one (82.4%) patients had no hypoplasia of IJV while 13 (17.6%) of them had ipsilateral hypoplasia of IJV. The group with hypoplastic IJV and the stroke on the same side, had TACI in 38.5% and PACI in 61.5% cases.

Right IJV was hypoplastic in 6 patients (41.7%), with the mean diameter of 4.4 mm. Left IJV was hypoplastic in 7 patients (58.3%) with the mean diameter of 4.2 mm. The mean diameter of hypoplastic IJV was  $4.4 \pm 0.5$  mm [mean  $\pm$  standard deviation (SD)], the median was 4.4 mm, the minimal diameter was 3.4 mm and the maximal was 5.0 mm. On

the contralateral side, the diameter was  $7.6 \pm 2.2$  mm (median 8.3 mm; minimum 3.5 – maximum 11.5 mm).

Comparing the clinical outcome in the group of patients with territorial infarction in anterior circulation and ipsilateral hypoplasia, we did not find a statistically significant difference. Similar percentage of patients in both group (without and with ipsilateral hypoplasia) had good or worse outcome. Four (20%) had mRS < 2 and 9 (16%) had mRS  $\geq 3$  ( $\chi^2$  test;  $p = 0.73$ ).

We found a statistically significant difference comparing the clinical outcome in the group of patients with territorial infarction in anterior circulation and unilateral ipsilateral hypoplasia IJV only on a stroke side. Nine (69.2%) patients with unilateral hypoplasia on a stroke side had poor outcome while only one (7.7%), in the group with good outcome had unilateral hypoplasia. (Fisher's exact test;  $p = 0.014$ ).

In the group of 13 patients with ipsilateral IJV hypoplasia, 4 (30.8%) patients had good outcome. Based on the NIHSS values for a stroke severity, 3 patients had mild and one patient had moderate stroke. Three patients with good clinical outcome had bilateral hypoplasia of IJV, whereas only one patient had unilateral hypoplasia with a small diameter of contralateral IJV (6.3 mm) (Table 2).

In the multivariate regression analysis as the outcome predictor in our study for the patients with AIS, we stressed out the infarction size (Table 3), and thus we separately evaluated the presence of IJV hypoplasia and the infarction size on the treatment outcome in the patients with TACI and PACI (Table 4). In the group of patients with TACI, there was a statistically significant difference in frequencies of the participants without hypoplasia, with unilateral and with bilateral TACI in relation for treatment outcome (Table 4), while in the group of patients with PACI, there were no statistically significant differences in the frequencies of participants regarding presence and side of PACI in relation for treatment outcome (Table 4).

**Table 2**

**Demographic and clinical features of patients with internal jugular vein (IJV) hypoplasia**

Patient No	Age (years)	Gender	Infarct side	Oxford scale	Etiology TOAST	NIHSS (admission)	AP Dmax ipsilateral IJV (mm)	AP Dmax contralateral IJV (mm)	mRs (discharge)
1	74	F	L	TACI	2	22	4.8	8.8	6
2	84	F	L	TACI	1	15	3.7	9.4	5
3	75	F	R	TACI	1	14	4.4	11.5	4
4	78	F	R	PACI	2	7	4.2	8.6	4
5	65	F	L	PACI	2	8	4.1	8.3	4
6	74	M	L	PACI	4	11	4.9	8.3	4
7	80	M	L	TACI	1	7	3.4	8.3	3
8	70	F	L	PACI	2	8	4.8	8.3	3
9	54	M	R	PACI	5	11	4.9	8.4	3
10	82	F	R	TACI	2	15	5	5	2
11	66	F	R	PACI*	2	3	4.1	6.3	1
12	73	M	L	PACI	1	6	4.6	4.3	1
13	74	F	R	PACI	5	4	4	3.5	1

**M – male; F – female; R – right; L – left; TACI – total anterior circulation infarct; PACI – partial anterior circulation infarct; \* – bilateral frontal stroke; TOAST – Trial of ORG 10172 in Acute Stroke Treatment: 1 – large-artery atherosclerosis; 2 – cardioembolism; 3 – small-artery occlusion-lacune; 4 – stroke of other determined etiology; 5 – stroke of undetermined etiology; NIHSS – National Institute of Health Stroke Scale; AP Dmax – anterior posterior maximal diameter IJV; mRs – modified Rankin Scale.**

Table 3

**Multivariate logistic regression analysis of the evaluated parameters and treatment outcome in the patients with the acute ischemic stroke**

Parameters	With/Without other conditions	
	ExpB (95% CI ExpB)	<i>p</i>
Alcohol	0.178 (0.021–1.492)	0.112
Ischemic area (TACI/PACI)	0.106 (0.012–0.945)	0.044*
NIHSS on admission	1.137 (0.971–1.330)	0.110

\**p* < 0.05; CI – confidence interval. For other abbreviations see under Table 2.

Table 4

**Distribution of treatment outcome frequencies regarding the type of ischemic area and presence of internal jugular vein (IJV) hypoplasia**

Ischemic area (IJV hypoplasia)	Outcome, n (%)		<i>p</i>
	good	poor	
TACI			
none	0 (0)	22 (100)	0.000
unilateral	0 (0)	4 (100)	
bilateral	1 (100)	0 (0)	
PACI			
none	16 (41.0)	23 (59.0)	0.113
unilateral	1 (16.7)	5 (83.3)	
bilateral	2 (100)	0 (0)	

\**p* < 0.05.

For abbreviations see under Table 2.

**Discussion**

In this study, we attempted to determine the influence of cerebral vascular congestion (CVC) on the outcome of an acute territorial ischemic stroke in anterior circulation, in terms of present hypoplastic jugular vein on the infarction side. We hypothesized that the cerebral venous congestion might influence the stroke outcome through impact on CPP or through disbalance of cerebrospinal fluid (CSF) resorption. It is known that CPP depends on the mean arterial and intracranial pressure, while the intracranial pressure resembles the cortical veins pressure<sup>2</sup>. Further, the cortical veins pressure depends on the pressure in IJV. In a supine position, IJV drain the largest portion of the blood from the brain. The ultrasound studies that evaluated anatomical characteristics of IJV showed that congenital small IJV or hypoplastic IJV were present in 10%–23% of the population<sup>4,5</sup>. Our findings are in correlation with the previous reports where we pointed out that 16.7% of the patients were diagnosed with hypoplastic IJV.

IJV that are of a small diameter decreased the blood volume flow with the consequent congestion in the intracranial venous system, since, the blood flow rate was shown to be directly proportional to the average velocity and the vein diameter<sup>11</sup>. Some studies showed that the right IJV diameter was wider, stressing out that right IJV was dominant particularly in 2/3 of the patients<sup>12,13</sup>. Our study correlates with previous reports since we found that in 63.5% of the evaluated patients the right IJV was of a wider diameter.

A great proportion of studies in last decade dealing with CVC was done on the patients with multiple sclerosis (MS), indicating as possible causes: neck veins with multifocal lu-

men narrowing, jugular reflux and jugular veins hypoplasia<sup>11,14</sup>. Further, it was stressed out that CVC was thought to be among the causes of leukoaraiosis and normotensive hydrocephalus<sup>15,16</sup>. Previous reports pointed out that in these neurological conditions, there was a chronic decreased reuptake of CSF in dural sinuses due to the elevated venous pressure, which lead to the presence of stasis in capillaries. These processes altogether directly influence CPP<sup>17</sup>. It was also noticed that the small IJV presence lead to chronic cerebral venous insufficiency and congestion<sup>11</sup>.

Some studies demonstrated that in an acute territorial infarction, on MRI, on the side of infarction, the deep medullar veins could be seen and that they were the predictors of a severe deficit according to the NIHSS and poor outcome<sup>18</sup>. Our findings stressed out that the patients with unilateral hypoplasia on the infarction side had poorer outcome then others. It should be pointed out as well, that the patients with bilateral hypoplasia had a satisfactory outcome in both groups (PACI and TACI). Our study demonstrated that the TACI patients with unilateral hypoplasia had poorer outcome, while one patient with bilateral hypoplasia had better outcome. This observation might be explained by the fact that in such a case there could be some kind of the venous blood flow reorganization from jugular to non-jugular (most likely to vertebral plexus) and thus preventing blood drainage from the brain misbalance, further preventing as well the cerebral venous congestion<sup>19</sup>. CVC in the acute cerebral infarction leads to an increased pressure in veins and capillaries, impairing the blood-brain barrier, that is already damaged by the acute ischemic stroke, leading to the vasogenic edema<sup>20</sup>. In addition, the cerebral venous congestion leads to a decrease in the perfusion pressure along with a reduction in the cerebral blood flow, causing further damaging of the brain tissue. The

deep medullary veins are dilated due to the venous insufficiency and congestion in the patients with leukoaraiosis<sup>21</sup>.

There are no studies with the ultrasonographic follow-up measurements of IJV in acute stroke. However, one angiographic study reported that the IJV and venous sinus hypoplasia may be the cause of the fatal edema in malignant media syndrome<sup>6</sup>.

This is in line with our results, where we stressed out that the presence of IJV hypoplasia on infarction side was associated with poorer prognosis, and bilateral hypoplasia with the better outcome.

Despite the obtained results from our study, we should stress out the study limitations. The main limitation refers to a sample size of the stroke patients with IJV hypoplasia. Therefore, further studies are needed to be conducted on a larger number of participants for more sensitive interpretation of treatment outcome in the patients with the acute myocardial infarct regarding the presence of unilateral or bilateral IJV hypoplasia.

## Conclusion

Ipsilateral, hypoplastic IJV is the cause of poor clinical outcome in the patients with the acute anterior circulation stroke due to reduced drainage of blood from the affected cerebral hemisphere. It is possible that in the acute phase, the venous circulation cannot adapt to the new situation fast enough, which further leads to an increase in the intracranial pressure, edema and a poor clinical outcome. In our study, the patients with bilateral hypoplasia of IJV had good clinical outcome. The main reason for this is probably a good adaptation of venous circulation to narrow IJV and enhanced drainage of the blood from the brain to non-jugular pathway most likely through the vertebral veins. The routine ultrasound examination of veins might be of a great importance in selection of the patients with territorial infarction who are expected to develop edema and thus progression of the neurological condition influencing poorer treatment outcome.

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## Comparative analgesic efficacy of ultrasound-guided nerve blocks induced by three anesthetics with different duration of action in the treatment of resistant neuropathic pain in the lower extremities

Poređenje analgetske efikasnosti blokova nerava pod kontrolom ultrazvuka pomoću tri anestetika različite dužine dejstva u lečenju rezistentnog neuropatskog bola donjih ekstremiteta

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### Abstract

**Background/Aim.** The neuropathic pain (NP) treatment is a big medical and socioeconomical problem. The new sorts of the NP treatment was developed and are applied in case of a medical treatment failure. The aim of this work was to investigate the efficacy of the ultrasound-assisted treatment of the resistant and chronic peripheral neuropathic pain with the local anesthetic nerve blocks. Due to the inefficacy of conventional treatment, three local anesthetics (short-acting, medium-term and long-acting) were administered in a series of the same minimal dose on a daily basis. Complications, side effects, the execution time of procedure and the onset time of local anesthetic were also investigated. **Methods.** In this prospective, randomized and double-blinded study, 108 patients (of which 53 were diagnosed with diabetes and 55 with radiculopathy) with the resistant and chronic peripheral neuropathic pain in the lower extremities were treated with a series of ultra-sound assisted peripheral nerve blocks. The conventional treatment was exhausted. The presence of this neuropathic pain was confirmed by, at least, one of the three scales – the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) scale, the Dolour Neuropathic 4 questions (DN4) scale and the pain DETECT(PD-Q) scale. Other therapies were not applied. The nerve blocks were administered on a daily basis until the relief of pain (visual analogue scale – VAS < 30), and after that, two additional nerve blocks were given. The three local anesthetics of the different duration of therapeutic effect were given at the same minimal dose: the short-acting (1% procaine-chloride solution), medium-term (1% lidocaine-chloride solution) and long-acting (0.25%

levobupivacaine-chloride solution) local anesthetics were used. The therapeutic efficacy was measured with the percentage reduction in the pain intensity on the VAS scale before and after the therapy and one month after the treatment: > 50% – excellent results; 31–49% – good results; < 30% the therapy did not work. The side effects, complications, the execution duration of procedure, the onset time of numbness, the number of corrections of the needle direction were recorded as well. **Results.** For all three groups: nerve blocks took  $5.4 \pm 1.48$  minutes to do (without difference among the groups), the onset of numbness occurred, on average, within  $3.75 \pm 2.62$  minutes (without differences among the groups), and the need for corrections of needle direction was minimal ( $1.03 \pm 0.17$  corrections). All the patients experienced a loss of pain sensation (VAS < 30); when a long-acting anesthetic was used, the number of required nerve blocks was significant ( $p < 0.001$ ) smallest ( $4.33 \pm 0.63$  blocks), than in other two groups, and the percentage pain reduction was highest (73.13%) ( $p < 0.001$ ). The pain relief lasted one month after the therapy without the application of any other therapy. Neither complications nor side effects were observed. **Conclusion.** The procedure described is a safe, efficient and easy-to-perform and does not lead to any complications and side effects. The pain relief is achieved most effectively and rapidly with the long-acting local anesthetics, and maintained even for one month without the introduction of any additional therapy.

**Key words:**  
neuralgia; nerve block; ultrasonography; lower extremity.

## Apstrakt

**Uvod/Cilj.** Lečenje neuropatskog bola (NB) je veliki medicinski i socioekonomski problem. Kada se iscrpe medikamentozne metode lečenja, razvijaju se novi pravci u lečenju NB. Cilj rada bio je ispitivanje efikasnosti lečenja hroničnog, rezistentnog perifernog NB blokovima lokalnih anestetika pod kontrolom ultrazvuka. Zbog neefikasnosti konzervativnog lečenja, primenjene su svakodnevne serije blokade tri lokalna anestetika (kratkog, srednjedugog i dugog dejstva), u istoj minimalnoj dozi. Beležene su komplikacije, neželjeni efekti, vreme potrebno za izvođenje procedure i započinjanje delovanja lokalnog anestetika. **Metode.** U ovom prospektivnom, randomizovanom i dvostruko-slepom istraživanju, 108 pacijenata (53 sa dijagnostikovanim dijabetesom i 55 sa radikulopatijom) sa rezistentnim i hroničnim perifernim NB u donjim ekstremitetima lečeno je serijom blokada perifernih nerava lokalnim anestetima, pod kontrolom ultrazvuka. Konzervativno lečenje je bilo iscrpljeno. Postojanje NB je potvrđeno najmanje jednom od tri skale – the *Leeds Assessment of Neuropathic Symptoms and Signs* (LANSS) skala, the *Douleur Neuropathique 4 questions* (DN4) skala i the *pain DETECT questionnaire* (PD-Q) skala. Drugo lečenje nije primenjivano. Blokade nerava su davane svakodnevno do postizanja obezboljavanja [Vizuelno-analogni skala (VAS) < 30], i još dve blokade nakon toga. Primenjena su tri lokalna anestetika različite dužine dejstva u istoj minimalnoj dozi: kratkog dejstva (1% rastvor prokain-hlorida), srednjedugog dej-

stva (1% rastvor lidokain-hlorida) i dugog dejstva (0,25% rastvor levobupivakain-hlorida). Terapijska efikasnost je merena procentom smanjenja bola merenog VAS pre i posle lečenja i jedan mesec nakon završetka terapije: odličan rezultat > 50%; dobar rezultat 31–49%; terapija ne deluje < 30%. Beleženi su i neželjeni efekti, komplikacije, trajanje procedure, vreme potrebno za razvoj trnjenja i broj korekcija pravca igle. **Rezultati.** Za sve tri grupe izvođenje nervnog bloka je trajalo  $5,04 \pm 1,48$  min (bez razlike između grupa), početak trnjenja je nastajao u proseku posle  $3,75 \pm 2,62$  min (bez razlike između grupa), a potreba korekcije pravca igle bila je minimalna ( $1,03 \pm 0,17$  korekcije). Svi pacijenti su bili obezboljeni (VAS < 30) kada je primenjen anestetik dugog dejstva; broj potrebnih blokada za taj efekat bio je visokoznačajno ( $p < 0,001$ ) manji ( $4,33 \pm 0,63$  blokada), nego u druge dve grupe, i procenat smanjenja bola bio je visokoznačajno veći (73,13%) ( $p < 0,001$ ). Gubitak bola se održavao mesec dana nakon završetka lečenja bez primene bilo kakve druge terapije. Nije bilo komplikacija, niti neželjenih efekata. **Zaključak.** Opisana procedura je bezbedna, efikasna i laka za izvođenje, nije praćena komplikacijama niti neželjenim efektima. Obezboljavanje se postiže efikasnije i brže sa anestetima dugog dejstva, i održava se jedan mesec bez bilo kakvog dodatnog lečenja.

## Ključne reči:

**bol, neuropatski; blokada živca; ultrasonografija; ekstremiteti, donji.**

## Introduction

The neuropathic pain (NP) is a pain arising as a consequence of the damage affecting the somatosensory part of the central nervous system (CNS) or peripheral nervous system. There are two types of the NP – central and peripheral<sup>1,2</sup>. By its nature, it is chronic (it lasts longer than three months, or could even last for years<sup>2</sup>), and it is commonly seen in the clinical practice (5% up to 20% of the general population suffer from the NP)<sup>2-4</sup>. The Canadian Association for the Study and Treatment of the Neuropathic Pain thinks that the annual amount of around 11 200 Canadian dollars is spent for the treatment of only one patient with the NP<sup>5</sup>.

The years 2014–2015 were declared as the Global Year Against the Neuropathic Pain to stress the importance of prevention, identification, treatment and socioeconomic severity of this problem<sup>6</sup>.

The neuropathic pain may occur in a single, or more often, in a mixed form<sup>7,8</sup>. The neuropathic pain component is diagnosed in up to 35% of all pain syndromes<sup>3,4</sup>: radiculopathy, the Failed Back Surgery Syndrome (FBSS), the pain in malignant diseases (particularly in bronchus), systemic and rheumatic diseases, the pain following the treatment with certain medications (for instance, chemotherapy), a part of the central pain (following an injury, surgery, ischemia, or the CNS infection), metabolic disorders (for instance, thyroid diseases), etc. It is essential to be familiar with specific questionnaires and ways to identify the neuropathic component of a mixed pain<sup>9-14</sup>.

According to the International Association for the Study of Pain (IASP) classification, one of typical forms of the NP is the peripheral neuropathy<sup>13</sup>. More than 100 types of peripheral neuropathy have been identified<sup>13</sup>, of which the diabetic neuropathy is very common – 60%–80% of patients with both type of diabetes may develop this form of the NP<sup>3,13</sup>.

The diabetic neuropathy is very similar to the nerve pain occurring after the Failed Back Surgery Syndrome (FBSS); when combined, they represent the most common form of the chronic peripheral neuropathic pain<sup>15</sup>.

Therefore, the painful diabetic neuropathy and radiculopathy with the neuropathic component is chosen as the model of chronic, localized peripheral NP for the, better understanding and identification of the NP as a component of a mixed pain.

The NP is diagnosed on the basis of the following: the confirmation that the nerve system was damaged by some agent; the overt manifestation of the damage and the identification of the typical somatosensory symptoms<sup>16,17</sup>. The presence of symptoms or signs only (for instance, allodynia or hyperpathia), does not justify the use of the term and diagnosis of the neuropathic pain<sup>15,16-18</sup>.

In practice, the presence of neuropathic pain component is the most easily identified by using several questionnaires such as the Leeds Assessment of Neuropathic Symptoms and Signs-LANSS scale<sup>12</sup>, *Douleur Neuropathique en 4 Questions* (DN4) scale<sup>9</sup>, Pain DETECT (PD-Q) scale<sup>14</sup>, which can detect the component of the NP. The questionnaires are the

most often used together in order to increase the accuracy of the NP detection in the course of the pain analyses.

The treatment efficacy is most often measured by the Visual Analogue Scale (VAS): the excellent result – > 50% pain reduction; a good result – 31%–49% pain reduction, the unsatisfactory result – < 30% pain reduction<sup>19</sup>.

All the pharmacological treatments were found to be ineffective in 20% to 40% of patients (non-responders) due to the common development of unacceptable side effects<sup>15, 20, 21</sup>. There is a great number of protocols for the neuropathic pain treatment that are recommended by the leading associations of the countries, pain societies and federations. The primary treatment of the NP is non-surgical – it is treated with a combination of several medications in 3–4 steps<sup>5, 22, 23</sup>. The first-line treatment involves the application of antidepressants and anticonvulsants, the local application of drugs often in combination with opioids that are most often considered the second or third-line treatment<sup>5, 24, 25</sup>. There is neither unique way for the NP treatment nor unique combination of medications for the same type of pain.

When the medical therapy is exhausted, the minimally invasive, interventional therapy is applied. It is any procedure requiring a small incision or a procedure during which the instruments are inserted into the body cavity reducing in that way the tissue damage to a minimum<sup>26, 27</sup>. The Special Interesting Group on Neuropathic Pain (NeuPSIG) was established within the framework of the International Association for the Study of Pain (IASP). According to the NeuPSIG definition, the interventional procedure is „an invasive procedure involving the delivery of drugs into the target location“<sup>15, 24</sup>. The high vitamin D doses, local anesthetics (LA), magnesium, gentamicin with or without corticosteroids are currently most commonly used in the NP treatment<sup>28–32</sup>. The success of the neuropathic pain management is the most frequently limited by the development of the unwanted effects<sup>29</sup>. Therefore, the local application of medications such as gels, plasters or injections has a significant place in the NP treatment<sup>17, 24, 25, 31, 32</sup>. During the application of a gel or plaster to the skin, a medication penetrates only 5 mm below the skin's surface; lidocaine and capsaicin can only be used in that way<sup>24</sup>. The USA and Germany have the longest experience in the application of LA in the form of gel or a plasters – eight years, and their application in the NP treatment was officially approved in some 50 countries<sup>24</sup>. On the other side, there is a much greater number of LA that can be used at any dose and dilution for the peripheral nerve blocks in the area where the NP is localized.

In addition to the needle prick, as disadvantages of the methods, the following were mentioned: the damage of a nerve or a blood vessel, nonselective effects of LA – the development of transient motor weakness, and, when very high doses are applied, cardiovascular and side effects of the CNS<sup>24, 31</sup>.

The recommendation of the NeuPSIG Group is to conduct investigations that could contribute to the precise refining of the nerve block protocols. This study was done in the accordance with this recommendation. The aim of the study was to investigate the efficacy of the ultrasound-guided treatment of the resistant chronic localized peripheral NP in the

lower extremities (LE) with three different local anesthetics – short-acting, medium-term and long-acting.

## Methods

A prospective, randomized, double-blinded, clinical study was conducted. The study included 108 patients divided into three groups. Three types of randomly chosen LA with the different duration of therapeutic effect were used for the nerve blocks: short-acting (1% procaine-chloride solution); medium-acting – (1% lidocaine-chloride solution), and long-acting a (0.25% levobupivacaine-chloride). The solutions of LA were prepared in the Military Medical Academy Pharmacy Sector, and were marked as the X1, Y1, Z1 layers – for double-blinded condition.

The inclusion criteria in the study were: the both genders; age > 18 years; the pain lasting longer than three months and shorter than six years; the presence of a resistant, chronic and localized peripheral NP in the lower extremities arising as a consequence of diabetes mellitus or as a neuropathic component of radiculopathy; the NP was confirmed by the scores on the LANSS pain scale:  $\geq 12$  scores or the pain DETECT scale:  $\geq 19$  scores or the DN4 scale:  $\geq 4$  scores; each patient filled-up each scale; the painful, lower-extremity diabetic neuropathy confirmed by a neurologist according to the valid recommendations of the 2010 European Federation of Neurological Societies (EFNS) guidelines<sup>15</sup>. The glycemic values were measured four times a day; in cases with this type of pain and radiculopathy in the lower extremities, radiculopathy was confirmed by the clinical, neurological and EMNG examinations; the previous pharmacological treatment was ineffective (VAS > 30), or side effects were unacceptable. All patients were mentally healthy and intellectually capable of understanding their participation in the study, and gave their informed consent.

The patients that were excluded from the study were those with ischaemic cerebral and/or myocardial diseases; metabolic mitochondrial diseases; liver diseases; acidosis; arrhythmias; hemorrhagic diathesis; psychiatric illnesses; epilepsy; organic CNS diseases confirmed by the magnetic resonance imaging (MRI); the allergic reaction to LA; the unregulated arterial hypertension. Other types of peripheral neuropathy detected through the adequate analyses, additional testings and examinations were also excluded.

The single nerve block therapy per day for the NP therapy was applied. Only one type of randomly chosen LA was given to one patient during the entire course of therapy. The nerve blocks were administered on a daily basis until the pain was released (VAS < 30 mm), two additional nerve blocks were given to determine therapeutic effects, but no more than ten nerve blocks were used. The subgluteal sciatic nerve blocks<sup>33</sup> (always with 5 mL of LA) and the lower inguinal lumbar plexus blocks<sup>33</sup> (the “3-in-1 block” always with 3 ml of LA) were used to pain therapy in the entire extremity, i.e., only the nerve block administered in the painful region, in the radiculopathy pain distribution with the same dose of LA.

The treatment efficacy was evaluated by the VAS scores before and after the pain relief and one month after the

completion of treatment. The evaluation was done in the following way: firstly, the VAS scores were measured before the therapy, at the end of therapy, and one month after the treatment. Then, the percentage of pain reduction was determined. The therapy results were scored as: excellent – 50% of initial pain reduction; good – 31%–49% of initial pain reduction; the therapy does not work – < 30% of initial pain reduction.

In addition to the VAS scores measured before and after the therapy as well as one month later, the onset of numbness (occurring simultaneously with the pain relief), were recorded by each patient (after daily examination of each patient, during the treatment and with the ultrasound examination before the new block).

The 8–18 MHz high frequency linear probe of the ultrasound machine, the screening program for peripheral nerves, and the B- and Color Doppler mode (on the Toshiba Aplio 500 Ultrasound Maschine) were used<sup>33</sup>. The blocks were performed by a specialist, trained for the ultrasound examination of the peripheral nerves, with the assistance of nurses. The execution duration of procedure, all side effects and complications, the number of corrections of the needle direction were recorded as well.

The Ethical Committee of the Military Medical Academy, Belgrade, Serbia, approved all the study procedures (Ethical Committee meeting dated November 30, 2015.).

#### Statistical analysis

The number of patients included into the study was based on the expected difference in the satisfactory pain relief results of three anesthetics. The minimally satisfying degree of analgesia was 30%, the statistic test power was 80% (0.08). Taking this into consideration (with statistic errors type 1) we calculated that the number of patients should be 36 per group making the total of 108 patients. The commercial statistical program GPower 3.1. was applied for the calculations.

Normality of the data was assessed by the Kolmogorov-Smirnov test. After that, the Friedman test, Wilcoxon Signed-Ranks test, the  $\chi^2$ -test, Mann-Whitney or Kruskal-Wallis test were used.

All the data were collected and processed using the SPSS program for Windows. They were presented in the standard way as the mean values with the standard deviation.

The value of < 0.05 was considered statistically significant, and the value of < 0.001 as highly significant and used for the multiple comparison tests.

#### Results

There were 3 groups of the patients treated with local anaesthetics, each consisted of 36 randomly chosen patients: the group 1 – the patients treated with 1% procaine-chloride solution (X1); the group 2 – the patients treated with 1% lidocaine-chloride solution (Y1) group and the group 3 – the patients treated with 0.25% levobupivacaine-chloride solution (Z1) group. The groups included roughly the same number of men and women ( $p = 0.65$ ), with similar mean age ( $p = 0.83$ ) and the neuropathic pain lasting, on average, for about 3 years ( $p = 0.74$ ). There was no significant differences ( $p = 0.75$ ) between the number of patients in the subgroups with diabetic neuropathy (DN) and radiculopathy with the neuropathic component (R) in all groups (Table 1).

There was a very significant difference among the groups in the number of nerve blocks (N) for the pain relief (VAS < 30): Kruskal-Wallis test,  $p < 0.001$ ; comparison of the N between the pairs performed by the Mann-Whitney test was  $p < 0.001$  in all cases. There was no difference between N for DN and R subgroups treatment in any of the groups (Wilcoxon test; for all results  $p > 0.05$ ). The very significant difference in N for the pain relief was found between all DN subgroups and all R subgroups, when different anesthetics were applied (the Kruskal-Wallis test and Wilcoxon test in all cases  $p < 0.001$ ).

The efficacy of anesthetics was measured by a level of pain with the VAS scale, before (VASp) the treatment, after the treatment (VASpp) and one month after treatment was completed (VASm). There was a very significant difference for all anesthetics ( $p < 0.001$ ). The positive trend in the pain relief continued in the groups 2 and 3 as well; in all cases the Friedman test showed statistically significant ( $p < 0.001$ ).

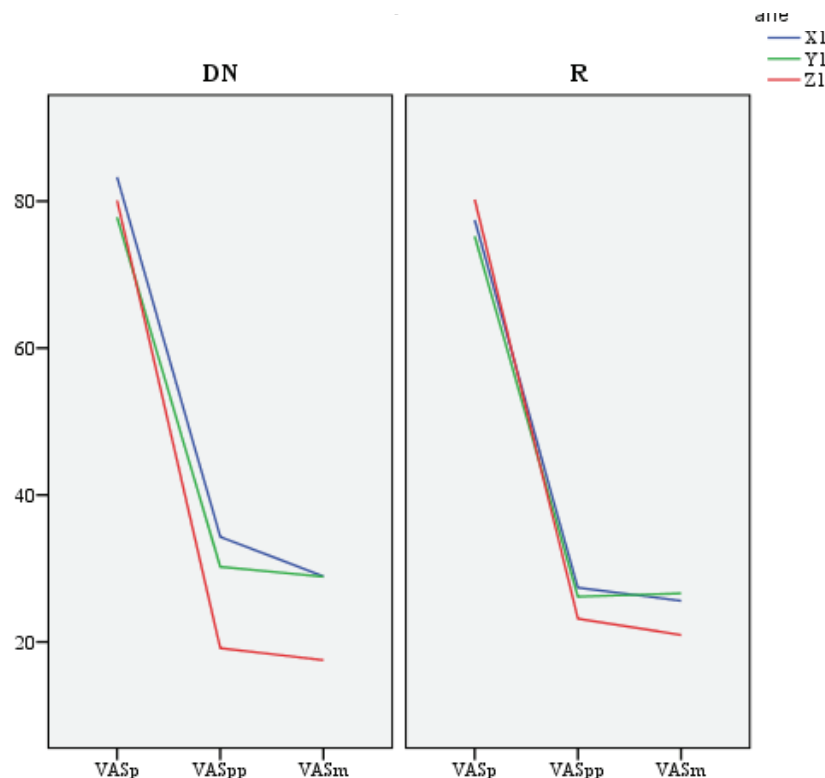
Table 1

**The efficacy of treatment with different anesthetics: the number of the blocks (N) for groups and subgroups treatment and level of the pain measured by Visual Analogue Scale (VAS) before the treatment (VASp), immediately after the treatment (VASpp) and one month after the treatment (VASm)**

Group/diagnosis	Blocks mean $\pm$ SD	VASp mean $\pm$ SD	VAS pp mean $\pm$ SD	VASm mean $\pm$ SD
Group 1	9.86 $\pm$ 0.54	80.06 $\pm$ 12.35	30.47 $\pm$ 9.26	27.08 $\pm$ 7.48
subgroup1 DN	10 $\pm$ 0	83.31 $\pm$ 8.65	34.31 $\pm$ 8.44	28.94 $\pm$ 8.61
subgroup1 R	9.75 $\pm$ 0.72	77.45 $\pm$ 14.35	27.40 $\pm$ 8.92	25.6 $\pm$ 6.28
Group 2	7.31 $\pm$ 2.28	76.53 $\pm$ 13.23	28.19 $\pm$ 7.60	27.75 $\pm$ 6.28
subgroup2 DN	7.33 $\pm$ 2.54	77.83 $\pm$ 14.18	30.22 $\pm$ 9.31	28.89 $\pm$ 6.05
subgroup2 R	7.28 $\pm$ 2.05	75.22 $\pm$ 12.48	26.17 $\pm$ 4.87	26.61 $\pm$ 6.47
Group 3	4.33 $\pm$ 0.63	80.17 $\pm$ 17.56	21.06 $\pm$ 7.35	19.14 $\pm$ 7.7
subgroup3 DN	4.37 $\pm$ 0.68	80.11 $\pm$ 15.87	19.16 $\pm$ 6.71	17.53 $\pm$ 7.27
subgroup3 R	4.29 $\pm$ 0.58	80.24 $\pm$ 19.78	23.18 $\pm$ 7.65	20.94 $\pm$ 7.97
Total DN	7.08 $\pm$ 2.76	80.30 $\pm$ 13.4	27.49 $\pm$ 10.33	24.83 $\pm$ 9.06
Total R	7.25 $\pm$ 2.58	77.58 $\pm$ 15.54	25.69 $\pm$ 7.48	24.49 $\pm$ 7.2

DN – diabetic neuropathy; R – radiculopathy.

Group 1 – patients treated with short-acting local anesthetic; Group 2 – patients treated with midium-acting local anesthetic; Group 3 – patients treated with high-acting local anesthetic.



**Fig. 1 – The efficacy of anesthetics in the subgroups with diabetic neuropathy (DN) and the subgroups with radiculopathy (R). X1 – short-acting local anesthetic; Y1 – medium-acting local anesthetic; Z1 – long-acting local anesthetic. For other abbreviations see under Table 1.**

There was no difference (measured by the VAS score) between the results of treatment subgroups DN and R within the same group, the Wilcoxon test showed  $p > 0.005$ , except for the group 3, immediately after the treatment ( $p = 0.039$ ); after one month, this difference disappeared ( $p > 0.05$ ) (Figure 1).

The very significant differences in the value of the VAS scores, were found between the same subgroups (DN and R) in all groups, when the different anesthetics were applied (Friedman and Wilcoxon test, in all cases  $p < 0.001$ ). The lowest values of the VAS score was achieved in the group 3. The trend of the excellent results continued one month after the therapy was accomplished in the subgroup with DN after the treatment with 1% procain-chloride solution and in the subgroup R after the treatment with 0.25% levobupivacaine-chloride solution ( $p < 0.05$ ) (Table 2).

The highest percentage in the pain reduction (73.73%) was achieved by the application of long-acting anesthetic in the group 3. The positive trend in the pain relief in relation to the VAS

scores measured before the treatment continued in all local anesthetic groups, but the highest trend was noticed in the group of long-acting local anesthetic (76.13%) (Table 3).

The nerve block procedure lasted for some five minutes on average, the onset time of numbness after the completion of procedure was less than four minutes ( $p > 0.05$ ).

**Table 2  
The average execution duration of procedure (in minutes), and the onset time of nerve block (NB) (in minutes)**

Anesthetics*	The duration of NB (mean $\pm$ SD)	The onset time of NB (mean $\pm$ SD)
Group 1	4.9 $\pm$ 1.52	3.78 $\pm$ 2.72
Group 2	5.1 $\pm$ 1.53	3.82 $\pm$ 2.58
Group 3	5.02 $\pm$ 1.68	3.65 $\pm$ 2.51
Total	5.04 $\pm$ 1.58	3.75 $\pm$ 2.62

\*For explanation see under Table 1.

**Table 3  
The percentage of the pain reduction measured by the Visual Analogue Scale (VAS) score: immediately after the treatment completed and immediately and one month after the treatment completed**

Anesthetics*	VAS score (% of initial pain reduction)		Assesment of results
	immediately after treatment completed	a month after treatment completed	
Group 1	62.06	66.23	excellent
Group 2	63.02	63.68	excellent
Group 3	73.73	76.13	excellent
Total	67.47	71.69	excellent

\*For explanation see under Table 1.

There were no serious complications (injuries of the nerves or vessels) or unwanted effects recorded even though  $7 \pm 2.7$  nerve blocks on average were administered to each patient (over 750 nerve blocks/108 patients). The only mild side effect that occurred occasionally was the development of subcutaneous hematomas not larger than 1 cm in diameter at the site of the needle insertion into the skin and subcutaneous tissue.

The average number of corrections of the needle direction was  $1.03 \pm 0.17$ .

## Discussion

The treatment of neuropathic pain is often inefficient – most often limited and caused by the development of side effects<sup>3,5,15</sup>. Therefore, new methods involving the local application of medications in the areas of the nerve structures that innervate the location where the pain is localized need to be found<sup>25</sup>.

The local application of medications in the neuropathic pain treatment may be non-invasive (topical), in a form of a gel or skin patch, and invasive – various nerve structure blocks and the instillation of medications into the body cavity where nerve structures are located<sup>3,5,15</sup>.

The development of non-invasive methods started in 1998, when a gel and skin patch with the 5% lidocaine-chlorid solution intended for the acute pain management was first produced in the USA. Nowadays, there are gels and skin patches containing the combination of 2.5% procaine-chlorid and 2.5% lidocaine-chlorid<sup>24</sup>. They are the only FDA recommendation for the neuropathic pain therapy in the post-herpetic neuralgia. Beside the USA, the Italian and German Chronic Pain Schools have the longest experience in their application (eight years). However, beside in the USA, the skin patches and gels are registered in 50 other countries. In the EU countries, they have been in use since 2008<sup>34–36</sup>. In addition to the fact that the patients cannot always access them officially, the patches and gels contain only lidocaine and procaine-type local anesthetic, which penetrate only five millimeters below the skin's surface, and that is why they are almost ineffective in the obese patients or in the areas of the deeper nerve structures<sup>34</sup>.

The therapy of chronic neuropathic pain has become more important over the recent years because it allows for the application of a larger number of different medications: higher doses of the vitamins D and D3, various local anesthetics, magnesium, gentamicin with or without corticosteroids, various concentrations, doses with the much greater accuracy and considerably smaller number of complications<sup>33,37</sup>.

The ultrasound guidance for the performance of the nerve blocks allowed for the reduction of applied doses and complications in particular. The description of the nerve structure and the execution of the nerve block procedure take place in real time, and thus, reduce the number of damages to the nerve structures and major blood vessels by some 30%<sup>32,33,38</sup>. Although the ultrasound was used for the first time in 1978, only two studies on that issue have been published until 2002, when, in the next year, that number am-

mounted up to 43<sup>38,39</sup>. The ultrasound-guided low-extremity nerve block was introduced much later in Germany, the country with one of the strongest associations for the ultrasound clinical application. This method has been used in the low-extremity treatment for the last six to seven years<sup>32,33,35</sup>. It is, therefore, not surprising that, in its current recommendation on the interventional treatment of neuropathic pain, the NeuPSIG stresses the need for further and more thorough study of peripheral nerve and plexus block protocols, as well as for defining their place, doses of medications to be used for such purposes and the execution protocols<sup>21</sup>.

There are not many studies devoted to this issue, the published series are very rare and insufficient for deriving serious conclusions taking into account that this is a very actual and still developing field.

Despite the fact that local anesthetics have, been used for a long time for the management of the acute pain during surgical procedures, the nerve blocks with local anesthetics have been recently introduced into the treatment of the chronic and neuropathic pain in particular. In the treatment of the acute pain during a surgery, the local anesthetics are also used for developing motor paralyses in the extremities, which explains the use of doses 60–200 times higher than those applied in our study and which proved to be sufficient to treat the outpatients with neuropathic pain<sup>32,33</sup>.

The occurrence of the motor paralyses in the outpatients with the neuropathic pain is not desirable, because, it is very disturbing for the patient who is even warned that it might happen and is transient. The motor weakness requires the hospitalization of the outpatients and their close monitoring, and in case of the chest muscle blocks, their vital functions should be monitored for at least two hours. Because of that, the motor weakness is not desirable and represent a side effect or even a complication.

Higher concentrations of LA accelerate onset of effect in the isolated nerve. The duration of the effect depends on the dosage and concentration of local anesthetic, resorption from tissue into the blood, and its building to the membrane receptors (protein-binding activity). To avoid motor weakness of a lower extremity, we applied smaller dose and concentration LA during the pain treatment study. The subgluteal and inguinal region were anatomically poorly vascularized, with low resorption into the blood consequently. The potency *in vitro* (isolated nerve) for procaine (X1), lidocaine (Y1) and levobupivacaine (Z1) is 1, 4, and 16 respectively. The protein binding for X1, Y1 and Z1 is 5.8%; 64%–70% and 97% respectively. The duration of a single-dose injection effect is 0.5–1 h; 2–4 h and 4–7 h for X1, Y1 and Z1 respectively. The duration of anesthesia, after a single-dose injection, is significantly longer with Z1 than with any other LA<sup>32</sup>.

Since the minimum dose of the local anesthetics was applied, no complications or side effects were observed in the course of our investigation. The pain relief was achieved by daily repetitive nerve blocks (the cumulative analgetic effect of local anesthetics<sup>32,33</sup>), the application of the local anesthetic directly to the surface of the nerve structure that caused the pain, and the use of the ultrasound based on the

knowledge of the ultrasound anatomy of the nerve and the nerve block area.

Thus, the patient experienced the pain relief immediately, simultaneously with the clinical sensation of numbness, because the sensitive nerve fibers are always grouped together.

The comparison of the three local anesthetic groups with the various duration of the therapeutic effect showed a greater efficacy of the long-acting local anesthetics: the pain relief was achieved by a smaller number of the nerve blocks, and the decline in the pain intensity was larger as compared with the use of the medium-term and short-acting local anesthetics ( $p < 0.001$ ). This effect is probably a consequence of longer de-excitation of the nociceptive and supraspinal systems and the achievement of balance between the nociceptive systems and activities of the antinociceptive pathways. However, further investigation in that direction is certainly needed<sup>33</sup>.

Certainly, we can discuss about achieving better efficacy of the local anesthetics because there were three groups of patients treated with different local anesthetics which had very similar mean VAS scores at the beginning of the study and they remained similar when compared them regarding the gender, age of patients, the number of patients with diabetes and radiculopathy in each group.

It is well-known that the mechanism of action of local anesthetics and antiepileptics is very similar –the target site of their action are voltage-dependent sodium channels. To fully achieve the effect of antiepileptic drugs, the continuous use of medications for four weeks is required<sup>15</sup>, and that is why we, in our study, re-evaluated the treatment efficacy after that period. Therefore, it is not surprising that the effectiveness of therapy in our study was even larger after four weeks, without the introduction of any additional therapy.

Based on this experience, we can stress that disadvantages of this method, without any doubt, are invasiveness and the patient's need for the daily visits which is particularly difficult for the patients living far away. Moreover, the method requires specific training – the knowledge about the ul-

trasound examination of peripheral nerves, the ultrasound anatomy and certain skills, because, if the in-plane technique is used, the one we applied in our study, the needle should always remain within the 1 mm wide beam from the ultrasound probe.

Based on the clinical experience, we suggested the application of the protocol involving daily administration of a minimal local anesthetic dose („3-in-1 block” with 3 mL, i.e., 5 mL of local anesthetic for the subgluteal sciatic nerve blocks), to prevent the possibility of side effects and development of transient paralyses of muscle groups in particular, since it requires the observation and hospitalization of a patient after the nerve block.

By performing the ultrasound-guided nerve blocks, we excluded the possibility of complications (the damage to a nerve or blood vessel). Having compared local anesthetics with different duration of therapeutic effect, we showed that a long-acting local anesthetic was the most effective allowing for the achievement of the pain relief in the patients after the smallest number of nerve blocks. On the other side, this treatment protocol requires patient's visits on a daily basis, represents an invasive and painful procedure that is gladly accepted by the patients for they feel pain relief almost after the first block. Therefore, this method may be applied in the treatment of the neuropathic pain only when all pharmacotherapeutic options are exhausted.

## Conclusion

The method that was applied in our study is efficient and easy-to-perform. No complications were observed.

Based on the percentage of the pain reduction and the smallest number of nerve blocks required to achieve the pain relief (VAS < 30), the long-acting local anesthetic was found to be the most efficient. Further investigation is required to highlight the mechanisms of pain relief, the cumulative effect of local anesthetics and the achievement of full effect for four weeks after initiating the therapy.

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## Spirometric changes in children with asthma exposed to environmental tobacco smoke and treated with inhaled corticosteroids

Promene spirometrijskih pokazatelja kod dece sa astmom koja su izložena duvanskom dimu i lečena inhalacionim kortikosteroidima

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### Abstract

**Background/Aim.** Corticosteroids are the most frequently prescribed anti-inflammatory treatment in asthma. A purpose of this study was to compare the spirometric parameters as a response to inhaled fluticasone propionate (FP) treatment in children with asthma, exposed and nonexposed to environmental tobacco smoke (ETS). **Methods.** The study included 527 children aged between 1 and 16 years with persistent asthma divided into the groups of ETS exposed (ETSE,  $n = 337$ ) and ETS free (ETSF,  $n = 190$ ) children. Spirometry was performed before (1st set of results) and after 6 months of FP treatment (2nd set of results). Good lung function (GLF) was defined as forced expiratory volume in one second ( $FEV_1 \geq 85\%$ ), and “poor lung function” (PLF) as  $FEV_1 < 85\%$ . **Results.** Among the ETSE children, 208 had one smoking parent, 129 had two, 228 had smoking mothers and 238 smoking fathers. The ETSE children received a higher FP dose ( $p < 0.0001$ ) which was increased with the increase of the number of smokers in the

family. The ETSE children had significantly lower lung function both in the 1st and 2nd sets of tests compared to the ETFS children ( $p < 0.05$ ). After the FP treatment, both groups improved all spirometric parameters ( $p < 0.001$ ). In the 2nd set of the spirometric tests, the children of smoking mothers had lower spirometry values compared to the children of smoking fathers ( $p < 0.05$ ). The proportion of the children improving from the PLF to GLF after 6 months of FP was much higher among the ETFS than the ETSE children ( $p < 0.05$ ). **Conclusions.** The ETSE children had lower spirometric values before FP. After 6-months of the FP treatment children in both groups improved the spirometric values, but the improvement was higher in the ETFS children.

### Key words:

asthma; child, preschool; child; adolescent; parents; smoking; tobacco smoke pollution; respiratory function tests; fluticasone; administration, inhalation.

### Apstrakt

**Uvod/Cilj.** Kortikosteroidi predstavljaju najčešće primenjivanu anti-inflamatornu terapiju u astmi. Cilj ovog istraživanja je bio da se uporede spirometrijski pokazatelji kao odgovor na terapiju inhalacionim flutikazon propionatom (FP) kod dece sa astmom, koja su izložena i koja nisu izložena duvanskom dimu (*environmental tobacco smoke* – ETS). **Metode.** Ispitivanjem je obuhvaćeno 527 dece uzrasta od jedne do 16 godina sa perzistentnom astmom, koja su podeljena u dve grupe: grupa izložena (exposed – E) duvanskom dimu (ETSE,  $n = 337$ ) i ETFS grupa – deca neizložena duvanskom dimu (*free* – F,  $n = 190$ ). Spirometrija je urađena pre propisivanja FP (1. set

rezultata) i nakon 6 meseci primene FP (2. set rezultata). Dobra plućna funkcija (*good lung function* – GLF) je definisana kao forsirani ekspirijumski volumen u prvoj sekundi ( $FEV_1 \geq 85\%$ ), a loša plućna funkcija (*poor lung function* – PLF) kao  $FEV_1 < 85\%$ . **Rezultati.** Među ETSE decom, 208 je imalo jednog roditelja pušača, 129 je imalo dva, 228 je imalo majku pušača, a 238 oca pušača. ETSE deci je propisana veća doza FP ( $p < 0,0001$ ), koja se povećavala sa povećanjem broja pušača u porodici. ETSE deca su imala značajno lošiju plućnu funkciju i u prvom i u drugom setu testova u odnosu na ETFS decu ( $p < 0,05$ ). Nakon lečenja sa FP, obe grupe su poboljšale sve spirometrijske pokazatelje ( $p < 0,001$ ). U drugom setu spirometrijskih testova, deca majki pušača imala su niže

vrednosti spirometrijskih pokazatelja u odnosu na decu čiji su očevi pušači ( $p < 0,05$ ). Procenat dece čija se plućna funkcija poboljšala od PLF do GLF nakon 6 meseci primene FP je bio mnogo veći među ETSE decom nego među decom 2. grupe ETSE ( $p < 0,05$ ). **Zaključak.** Parametri plućne funkcije izmereni pre propisivanja FP su lošiji kod dece izložene duvanskom dimu, nego kod dece koja nisu izložena. Nakon šestomesečne primene FP poboljšani su parametri plućne

funkcije kod obe ispitivane grupe, ali znatno više kod dece koja nisu bila izložena duvanskom dimu.

#### Ključne reči:

**astma; deca, predškolska; deca; adolescenti; roditelji; pušenje; zagađenje duvanskim dimom; respiratorna funkcija, testovi; flutikazon; inhalaciona primena.**

## Introduction

Asthma, as the most frequent chronic disease in children, could be triggered by infection, allergens, psychological and hormonal factors, physical exercise as well as by environmental irritants and contaminants such as tobacco. A retrospective analysis of the data from 192 countries, done by Oberg et al.<sup>1</sup>, showed that as many as 40% of children were regularly exposed to secondhand smoke. The World Health Organization (WHO), California Environmental Protection Agency, and the US Surgeon General Report have presented evidence of a higher incidence of the acute lower respiratory infections and acute otitis media, more hospital admissions and earlier onset of asthma in the environmental tobacco smoke (ETS) exposed children<sup>2-4</sup>. Many studies have provided evidence that a fetal exposure to the chemical mediators secondarily released in response to tobacco and postnatal passive smoking present a risk factor of reduced lung function<sup>5-10</sup>. The prenatal and postnatal effects of secondhand smoke may vary among individuals depending on the individual genetic susceptibilities and gene-environment interactions.

Corticosteroids are the most effective anti-inflammatory therapy for asthma. Several studies in adult asthmatics and chronic obstructive pulmonary disease (COPD) patients provided evidence that chronic inflammation in the airways of smokers may be resistant to the anti-inflammatory effects of corticosteroids<sup>11-15</sup>. Studying children, Cohen et al.<sup>16</sup> found that the ETS exposure may attenuate the beneficial effect of inhaled corticosteroids (IC) among the children with asthma.

Therefore, we conducted a study in order to: 1) assess whether the children with persistent asthma exposed to the ETS had the lower values of the lung function tests compared to the nonexposed ones, 2) examine if there was a difference between exposure to smoking mother or smoking father or to both smoking parents and 3) compare the effect of the inhaled corticosteroid treatment with the fluticasone propionate (FP) on the lung function parameters in the ETS exposed and nonexposed children with asthma.

## Methods

### Study design

This cohort study was conducted at the Children's Hospital for Respiratory Diseases and Tuberculosis, Belgrade, Serbia, from June 2011 to June 2012. We screened 726 children (6–16 years old) with persistent asthma, with one or more asthma

exacerbations treated in the emergency unit in the preceding 12 months, not receiving systemic or IC for at least two months. The diagnosis of asthma was established according to the Global Initiative for Asthma (GINA) recommendations<sup>17</sup>. The lung function testing was performed by means of spirometry (Pneumoscreen, Jaeger) and according to the protocol of the European Respiratory Society<sup>18</sup>. The subjects were suspended from the use of short-acting  $\beta_2$  adrenergic agents at least 6 hours before the test. The following parameters were measured: vital capacity (VC), forced volume vital Capacity (FVC), forced expiratory volume during the first second ( $FEV_1$ ),  $FEV_1/VC$ , peak expiratory flow (PEF), mean expiratory flow at 75% ( $MEF_{75}$ ), mean expiratory flow at 50% ( $MEF_{50}$ ), mean expiratory flow at 25% ( $MEF_{25}$ ) and mean expiratory flow at 25%–75% ( $MEF_{25-75}$ )<sup>19</sup>. Spirometry was performed at the beginning of the study (1st set of results) and after 6 months of the IC treatment (2st set of results). We classified the asthma severity according to the baseline value of the lung function parameters rather than according to the intensity and frequency of asthma symptoms<sup>20</sup>. According to the  $FEV_1 < 85\%$  or  $\geq 85\%$  of predicted values by age, sex, height and weight, we divided children into groups with the poor lung function (PLF) and good lung function (GLF). This cutoff point was chosen in order to define the group of asthmatic children with the lowest 5% lung function<sup>5</sup>.

Smoke free families and families with smoking parents (mother or/and father) were included into the study. Children who were active smokers themselves or had additional chronic illness such as nephritic syndrome, diabetes, epilepsy, etc., were not included in the study.

A FP metered dose inhaler (MDI) was used in the daily dosage ranging from 50 to 1,000 mcg for 6 months. The clinicians prescribed the initial FP dose according to the asthma symptoms, lung function parameters and patients' age. They were blinded to the child's ETS exposure. The dose was maintained through the 6 months of the study. The children below the age of 12 were instructed to use the FP MDI through the volumatic chamber. No one was treated with the long-acting  $\beta_2$  adrenergic agents or anti-leukotriene agents. An allergy and asthma history was obtained at the first visit. According to the responses and parental personal statements, we divided children into two groups: children exposed to ETS at home (exposed cohort – ETSE) and ETS free children (control cohort – ETSEF), and scheduled for the regular check-ups every 2 months. The skin prick test (SPT) was performed and the total serum IgE was taken to all children<sup>21</sup>.

The study was approved by the institutional review board of the Children's Hospital for the Respiratory Diseases

and Tuberculosis, Belgrade, Serbia and informed consents were obtained from the patients and parents/caregivers.

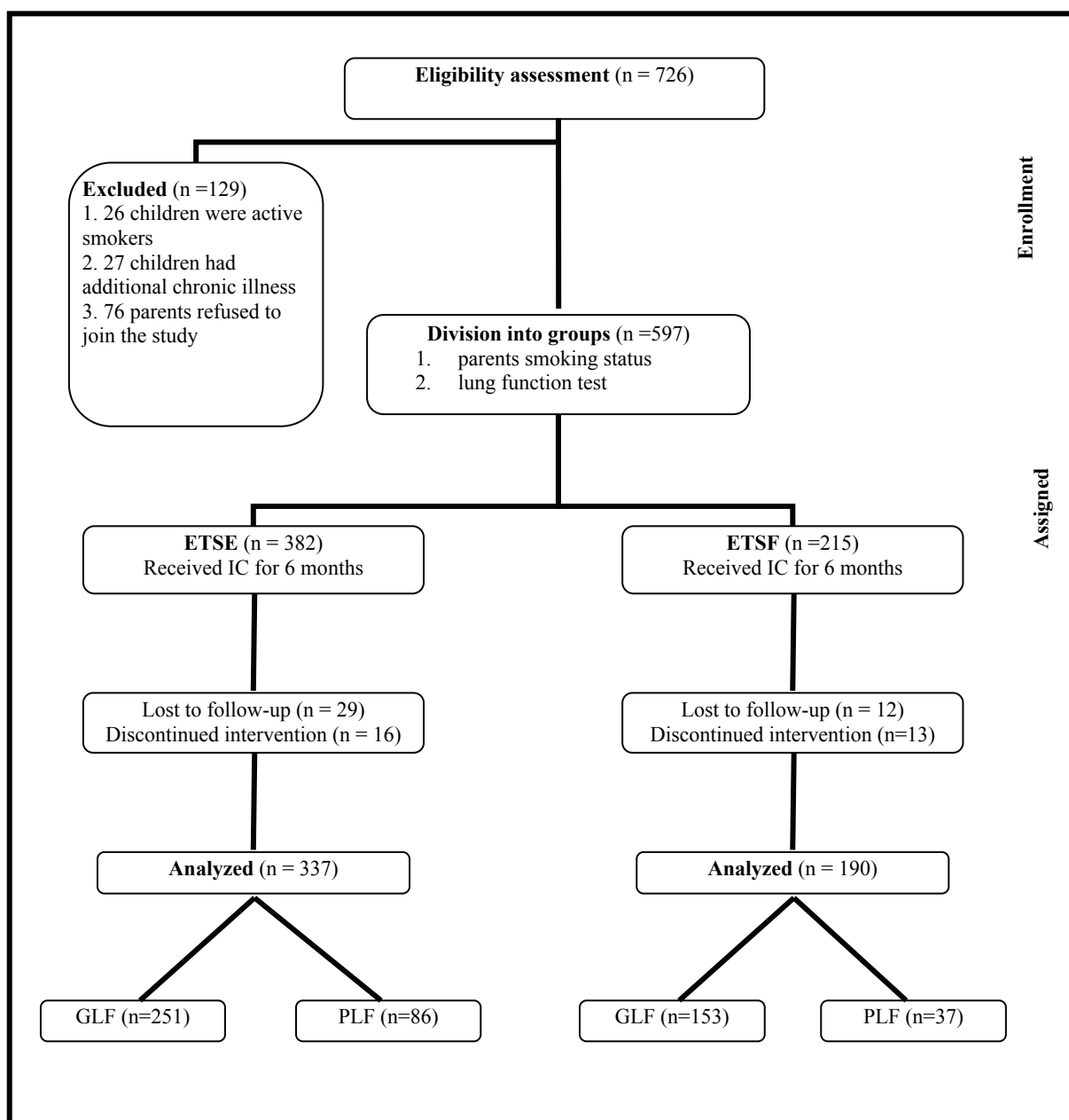
### Participants

Out of 726 children, we excluded 129 for not meeting the entry criteria.

The remaining 597 children were divided into the ETSE,  $n = 382$  (64%) and the ETSF,  $n = 215$  (36%) groups.

Additional 45 children were excluded from the ETSE group due to acute asthma exacerbation, lost contact, etc., leaving a sample of 337 children. We divided the ETSE children into the three subgroups: children of smoking mothers ( $n = 228$ ), children of smoking fathers ( $n = 238$ ) and children of both smoking parents ( $n = 129$ ).

From the ETSF group, 25 children were lost for reasons similar to the ETSE group, leaving the sample of 190 children (Figure 1, patients enrolment flow chart).



**Fig. 1– Patients enrolment flow chart.**

**ETSE – environmental tobacco smoke (ETS) exposed children;**

**ETSF – environmental tobacco smoke (ETS) free children**

**GLF – good lung function –  $FEV_1 \geq 85\%$  of predicted;**

**PLF – poor lung function –  $FEV_1 < 85\%$  of predicted.**

### Statistical analysis

All data was statistically analyzed using the software SPSS 17 for Windows. The ordinal data was analyzed by the Pearson  $\chi^2$  test and the Fisher exact test if needed. The odds ratio (OR) with 95% confidence interval (CI) were calculated from 2\*2 tables. The continuous variables are expressed as means  $\pm$  SD. The numerical data (after verification of normal distribution by the Kolmogorov-Smirnov test) in two groups were compared with the Student *t*-test for related samples (since we had only 2 time points), and Fisher parametric ANOVA (Analysis of variance) for comparing more than two groups or more than two measures. Homogeneity of variance was checked for all variables and in all cases *p*-values exceeded 0.05. The Fishers Least Significant Difference (LSD) test was used for multiple comparisons. The results were controlled by the Tukey test that provided identical *p*-values in all instances. A probability value of *p* < 0.05 was considered statistically significant.

### Results

In our sample of 527 children, there were 263 (49.9%) boys and 264 (50.1%) girls. There were 190 (36.1%) children with asthma living in the smoke free families, 208 (39.5%) living in the families with one smoking parent, and 129 (24.5%) living with two. There were 228 (43.3%) children living with actively smoking mothers (before the pregnancy 51% of mothers were smokers, during the pregnancy 41% continued to smoke) and 238 (45.2%) with actively smoking fathers.

In the first spirometry, the mean FEV<sub>1</sub> was 99.4%  $\pm$  16.4%. There were 432 (82%) children with asthma with FEV<sub>1</sub>  $\geq$  85% of the predicted value.

There was no difference between the ETSF and ETSE groups according to sex, age, weight and height. However,

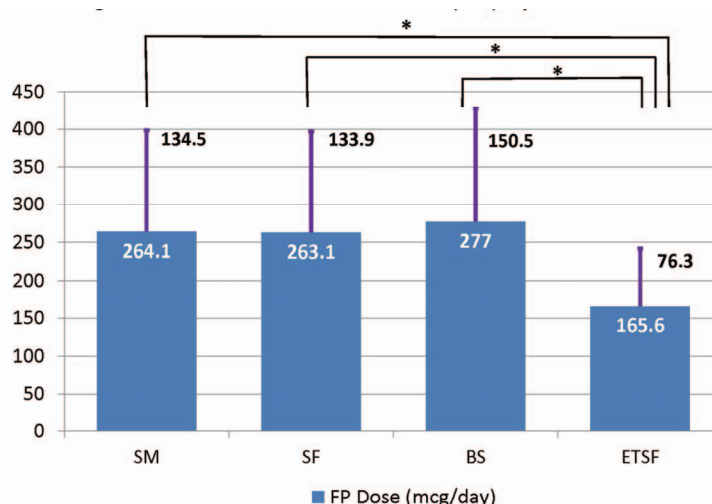
the significant differences were recorded in the following baseline parameters: FEV<sub>1</sub> (101.9 vs. 98.2 respectively; *t* = 2.47, *p* = 0.014), total IgE values (320.9 vs. 603.6 IU/mL respectively; *t* = 5.534, *p* < 0.0001), at least one positive SPT (57.3% vs. 76.5%,  $\chi^2$  = 20.7; *p* < 0.0001) and the number of asthma exacerbations in the preceding 12 months (5.3  $\pm$  4.2 vs. 7.54  $\pm$  10.7 respectively; *t* = 2.75, *p* = 0.006). The number of children, with FEV<sub>1</sub> < 85% of predicted, was significantly higher among smoking mothers ( $\chi$  = 6.0, *p* < 0.05), fathers ( $\chi$  = 7.8, *p* < 0.05) and both parents ( $\chi$  = 7.6, *p* < 0.05).

The arithmetic mean of the FP dose prescribed to all children was 225.1  $\pm$  120.0 mcg per child per day. The ETSE received a significantly higher FP dose (Figure 2) than the ETSF children (*F* = 31.2, *p* < 0.001). There was no difference in the FP dose between the subgroups of the ETSE children.

According to the FEV<sub>1</sub>  $\geq$  85% and FEV<sub>1</sub> < 85% of the predicted values and in the first spirometry and exposure to the ETS, we divided children into the subgroups: in the GLF group, there were 251 ETSE and 153 ETSF children, and in the PLF group there were 86 ETSE and 37 ETSF children. In the GLF group, the ETSE children had more exacerbations 12 months before the study, and a higher value of total IgE. They received a higher dose of the FP per day and had a higher percentage of at least one positive skin prick test compared to the ETSF children. In the PLF group, ETSE children had the higher value of total IgE and received a higher dose of FP compared to the ETSF children (Table 1).

All subgroups of the ETSE children had the significantly lower baseline values of all lung function test parameters than the ETSF children (Table 2).

In the final spirometry, the mean FEV<sub>1</sub> was 109.9  $\pm$  14.2%. All ETSF and ETSE children significantly improved all lung function parameters 6 months after the introduction of the FP, except for the FEV<sub>1</sub>/FVC values in the children of smoking mothers (*p* = 0.18).



\* *p* < 0.01, SM = Smoking Mother, SF = Smoking Father, BS = Both Smoking Parents, ETSF = Environmental Tobacco Smoke Free children

**Fig. 2 – Mean values of daily dose of fluticasone propionate (FP) (mcg) according to the child's environmental tobacco smoke (ETS) exposition.**

Table 1

## Baseline characteristics of children with asthma

Characteristics	Good lung function*		Poor lung function†	
	ETSE (n = 251)	ETSF (n = 153)	ETSE (n = 86)	ETSF (n = 37)
Age (years), mean ± SD	10.0 ± 2.8	10.5 ± 2.9	10.6 ± 2.9	10.2 ± 2.8
Male sex (n, %)	141 (56.2)	85 (55.6)	42 (49.4)	17 (45.9)
Weight (kg), mean ± SD	43.7 ± 15.2	42.5 ± 15.9	34.4 ± 12.2	35.3 ± 11.6
Height (cm), mean ± SD	147.9 ± 17.5	147.6 ± 18.2	140.6 ± 16.9	139.0 ± 14.1
Numbers of exacerbations in last 12 months, mean ± SD	7.8 ± 12.1	5.1 ± 3.9	6.6 ± 5.6	6.9 ± 5.8
IgE (IU/mL), mean ± SD	641.9 ± 613.5	335.2 ± 6.9 <sup>‡</sup>	518.6 ± 569.0	271.9 ± 361.2 <sup>‡</sup>
FP (mcg)/ day, mean ± SD	246.8 ± 112.2	169.9 ± 85.4 <sup>‡</sup>	245.6 ± 130.0	158.8 ± 95.8 <sup>‡</sup>
SPT (n, %)	200 (79.7)	84 (54.9) <sup>‡</sup>	66 (77.6)	27 (73)

ETSE – Environmental tobacco smoke (ETS) exposed children; ETFS – ETS free children; \*Good lung function – FEV<sub>1</sub> ≥ 85% pred; †Poor lung function – FEV<sub>1</sub> < 85% pred; SPT – Children with positive at least one skin prick test; \**p* < 0.05 and †*p* < 0.01 (Student's *t*-test or  $\chi^2$  test), as compared with the respective ETSE; SD – standard deviation.

Table 2

## Lung function tests parameters between the children of non- smoking and smoking mothers, fathers, one and both parents, taken before (1st spirometry) and after 6 months (2nd spirometry) of the fluticasone propionate (FP) treatment

Parameter	Smoking	1st Spirometry			2nd Spirometry		
		mean ± SD	LSD	<i>p</i>	mean ± SD	LSD	<i>p</i>
FEV <sub>1</sub> (%pred)	ETSF	102.6 ± 15.4	ETSF–SM	< 0.001	108.9 ± 15.4	ETSF–SM	< 0.001
	SM	96.5 ± 15.7	ETSF–SF	< 0.001	102.9 ± 15.7	ETSF–SF	< 0.05
	SF	97.9 ± 17.1	ETSF–BS	< 0.001	105.8 ± 17.1	ETSF–BS	< 0.01
	BS	96.2 ± 15.6	SM–SF	> 0.05	104.4 ± 15.6	SM–SF	< 0.05
			SM–BS	> 0.05		SM–BS	> 0.05
			SF–BS	> 0.05		SF–BS	> 0.05
FEV <sub>1</sub> /FVC (%pred)	ETSF	109.2 ± 9.4	ETSF–SM	< 0.001	110.5 ± 9.4	ETSF–SM	< 0.001
	SM	105.7 ± 11.5	ETSF–SF	< 0.05	106.5 ± 11.5	ETSF–SF	> 0.05
	SF	107.2 ± 10.5	ETSF–BS	< 0.01	109.1 ± 10.5	ETSF–BS	< 0.01
	BS	106.1 ± 11.9	SM–SF	> 0.05	107.7 ± 11.9	SM–SF	< 0.05
			SM–BS	> 0.05		SM–BS	> 0.05
			SF–BS	> 0.05		SF–BS	> 0.05
PEF (%pred)	ETSF	91.3 ± 17.0	ETSF–SM	< 0.001	96.7 ± 17.0	ETSF–SM	> 0.05
	SM	85.4 ± 16.7	ETSF–SF	< 0.01	94.7 ± 16.7	ETSF–SF	> 0.05
	SF	86.6 ± 17.5	ETSF–BS	< 0.001	97.9 ± 17.5	ETSF–BS	> 0.05
	BS	84.7 ± 16.2	SM–SF	> 0.05	97.7 ± 16.2	SM–SF	< 0.05
			SM–BS	> 0.05		SM–BS	> 0.05
			SF–BS	> 0.05		SF–BS	> 0.05
MEF <sub>25</sub> (%pred)	ETSF	93.8 ± 21.3	ETSF–SM	< 0.001	99.2 ± 11.3	ETSF–SM	< 0.001
	SM	82.2 ± 20.7	ETSF–SF	< 0.001	92.9 ± 10.7	ETSF–SF	> 0.05
	SF	85.5 ± 19.7	ETSF–BS	< 0.001	98.5 ± 13.7	ETSF–BS	> 0.05
	BS	83.0 ± 20.3	SM–SF	> 0.05	96.9 ± 10.3	SM–SF	< 0.01
			SM–BS	> 0.05		SM–BS	> 0.05
			SF–BS	> 0.05		SF–BS	> 0.05
MEF <sub>50</sub> (%pred)	ETSF	94.0 ± 24.3	ETSF–SM	< 0.001	100.8 ± 14.3	ETSF–SM	< 0.001
	SM	80.7 ± 22.5	ETSF–SF	< 0.001	91.9 ± 12.5	ETSF–SF	< 0.05
	SF	84.0 ± 21.5	ETSF–BS	< 0.001	96.8 ± 11.5	ETSF–BS	< 0.01
	BS	81.2 ± 22.1	SM–SF	> 0.05	93.4 ± 12.1	SM–SF	< 0.05
			SM–BS	> 0.05		SM–BS	> 0.05
			SF–BS	> 0.05		SF–BS	> 0.05
MEF <sub>75</sub> (%pred)	ETSF	94.9 ± 32.8	ETSF–SM	< 0.001	105.4 ± 12.8	ETSF–SM	< 0.001
	SM	80.5 ± 29.9	ETSF–SF	< 0.001	89.5 ± 19.9	ETSF–SF	< 0.05
	SF	81.7 ± 26.9	ETSF–BS	< 0.001	99.6 ± 16.9	ETSF–BS	< 0.01
	BS	81.0 ± 27.0	SM–SF	> 0.05	93.5 ± 17.0	SM–SF	< 0.01
			SM–BS	> 0.05		SM–BS	> 0.05
			SF–BS	> 0.05		SF–BS	> 0.05

ETSF – environmental tobacco smoke (ETS) free children (n = 190); SM – smoking mother (n = 228); SF – smoking father (n = 238); BS – both smoking parents (n = 129); LSD – Fisher's Least Significant Difference test for multiple comparisons; SD – standard deviation; FEV<sub>1</sub> – forced expiratory volume during first second; FVC – forced expiratory volume; PEF – peak expiratory flow; MEF<sub>25</sub> – mean expiratory flow at 25% of forced vital capacity; MEF<sub>50</sub> – mean expiratory flow at 50% of forced vital capacity; MEF<sub>75</sub> – mean expiratory flow at 75% of forced vital capacity.

The analysis of the 2nd set of spirometry results in Table 2 showed that: 1) the values of FEV<sub>1</sub>, MEF<sub>50</sub> and MEF<sub>75</sub> were still lower in all subgroups of the ETSE children compared to the ETSF children; 2) the value of FEV<sub>1</sub>/FVC was lower in the children of smoking mothers and both smoking parents compared to the ETSF children while there was no difference between the children of smoking fathers and the ETSF children; 3) the value of MEF<sub>25</sub> was lower in the children of smoking mothers compared to the ETSF children, however, there was no difference in the children of smoking fathers and both smoking parents compared to the ETSF children; 4) the children of smoking mothers had lower values of all lung function test parameters compared to the children of smoking fathers; 5) there was no difference in PEF between the ETSE and ETSF children.

There was no difference in FVC between the ETSF and ETSE children both in the 1st and 2nd sets of tests.

The improvement in IC was quite similar between the ETS exposed and not exposed children except in a few points. In the children of non-smoking mothers, an improvement of MEF<sub>25</sub> was significantly higher ( $p < 0.05$ ) compared to the children of smoking mothers. The children of smoking fathers had a significantly better improvement in MEF<sub>75</sub>, MEF<sub>50</sub> and MEF<sub>25</sub> ( $p < 0.05$ ) compared to the children of non-smoking fathers. The children of both smoking parents had a significantly better improvement in PEF and MEF<sub>75</sub> ( $p < 0.05$ ) compared to the children of both non-smoking parents (Table 3).

The comparison of FEV<sub>1</sub> according to the ETS exposure in the GLF and PLF children before and after the FP treatment estimated the risk of having poor lung function (Table 4). The percentage of children improving from the PLF to GLF after 6 months of the FP treatment was much higher in the ETSF than in the ETSE children. In the ETSE group, 51% of children passed from the PLF to GLF group. In the ETSF group, 82% of children passed from the PLF to GLF.

## Discussion

In this study a high percentage of smoking families (63.9%) was found, which is common in developing countries<sup>22-25</sup>.

Baseline characteristics of the ETSE children showed that they had lower FEV<sub>1</sub>, higher total IgE, more positive SPT and more previous asthma exacerbations compared to ETSF children. The ETSE children received a significantly higher FP dose per day. We assume that the clinicians prescribed higher doses of IC to the patients with more intensive symptoms and lower spirometry parameters, regardless of the child's tobacco exposure.

In this study, children with FEV<sub>1</sub> < 85% had more smoking parents<sup>6</sup>. In the GLF group, the ETSE children had more exacerbations 12 months before the study, had higher serum IgE values, received higher doses of FP per day, and had a higher percentage of at least one positive SPT compared to the ETSF children. In the PLF group, the ETSE children had higher the total IgE level and received a higher dose of FP per day compared to the ETSF children. Further analysis is required to determine whether

the increased rate of exacerbations was linked with the IgE status and not the ETS status. Another question is whether the ETSE children additionally suffered from another respiratory problem (COPD type). Many studies confirmed that children exposed to tobacco smoke had more exacerbations which are red flags of poor control<sup>1-4, 9, 10, 26</sup>. Some studies have documented an association between maternal smoking during pregnancy and elevated cord blood total IgE as well as an increased risk for the development of some allergic disease<sup>27, 28</sup>. Other studies, however, did not replicate these findings. The updated meta-analysis of the evidence relating parental smoking to allergic sensitization in children as measured by SPT, the IgE levels, and presence of allergic rhinitis and eczema did not show any significant association of maternal smoking with the total serum IgE, allergic rhinitis, or eczema, and had no effect on secondhand smoke on skin-prick positivity<sup>29, 30</sup>. Farooqi and Hopkin<sup>31</sup> suggested that there was no significant association between maternal smoking and atopy, and maternal atopy constitutes the main risk for the development of atopy in children. This study was conducted in children with asthma, and a significant difference was shown in the total serum IgE and positive SPT between the ETSE and ETSF children.

As we expected, the lung function test parameters in the ETSE and ETSF children significantly improved on IC. However, the lung function parameters in the ETSE children never reached the values of those in the ETSF children. What we found interesting was that the children of smoking mothers had lower values of all lung function test parameters in the final spirometry compared to the children of smoking fathers. This was probably due to the amount of the time smoking mothers spend at home with children and consequently the higher ETS exposure. Our findings confirmed the findings of many other studies that reported differences in the lung function between the children with asthma exposed and nonexposed to the ETS<sup>1, 6, 8</sup>. An international study of parental smoking and lung function in more than 20,000 primary school children showed the lasting effect of smoking during pregnancy on the lung function, while the effects of past and current ETS exposure were smaller<sup>5</sup>. Other studies exploring the effects of ETS on asthma severity showed that the ETS exposed individuals had worse lung function, more exacerbations, more health care resource utilization, and a higher level of bronchial hyperreactivity in comparison to those who were not exposed<sup>6-10</sup>.

The comparison the 1st and the 2nd set of spirometric results in the ETSF and ETSE children showed that some lung function parameters improved more in the ETSF and some in the ETSE children. Practically, the improvement was similar in both groups, although the ETSE children took higher doses of FP. This finding is in contrast with studies providing evidence that the ETS exposure may attenuate the beneficial effect of IC among children with asthma<sup>16</sup>.

The proportion of children improving from the PLF to GLF after 6 months of FP was much higher among the ETSF children compared to the ETSE children. This is in accordance with the work of Cohen et al.<sup>16</sup> who found that fetal exposure to chemical mediators secondarily released in response to tobacco impaired the response to inhaled corticosteroid in children with asthma.

**Table 3**  
**Comparison of improvement between the 1st and 2nd spirometry in the children of non-smoking and smoking parents (mothers, fathers and both parents),**  
**(two-way ANOVA)**

Parameter	Spirometry	Smoking	Smoking mother (n = 228)				Smoking father (n = 239)				Both smoking parents (n = 129)			
			Mean	SD	d*		Mean	SD	d*		Mean	SD	d*	
FEV <sub>1</sub> (% pred)	1st	no	101.6	16.7			100.6	15.7			100.4	16.6		
	2nd		108.1	13.2	6.5		106.0	14.5	5.4		106.2	14.2	5.8	
	1st	yes	96.5	15.7			98.0	17.2			96.4	15.7		
FEV <sub>1</sub> /VC (% pred)	2nd		102.9	14.9	6.4		105.7	13.7	7.7		104.7	14.0	8.3	
	1st	no	109.0	9.1	1.6		107.9	10.2	0.7		108.1	9.7	1.1	
	2nd		110.6	7.8			108.6	9.1			109.2	8.7		
PEF (% pred)	1st	yes	105.7	11.5	0.8		107.2	10.5	1.9		106.1	11.9	1.7	
	2nd		106.5	10.2			109.1	9.1			107.8	10.1		
	1st	no	90.4	17.7	6.8		89.6	17.3	5.0		89.3	17.6	6.2	
MEF <sub>75</sub> (% pred)	2nd		97.2	15.4			94.6	16.5			95.5	16.3		
	1st	yes	85.5	16.8	9.2		86.7	17.5	11.3		85.0	16.4	13.0*	
	2nd		94.7	17.4			98.0	15.9			98.0	16.1		
MEF <sub>75</sub> (% pred)	1st	no	91.9	20.5	7.5		89.5	22.1	5.6		89.2	21.2	7.2	
	2nd		99.5	18.4			95.1	21.5			96.4	20.6		
	1st	yes	82.3	20.7	10.6		85.6	19.7	12.9*		83.2	20.5	14.1*	
MEF <sub>50</sub> (% pred)	2nd		92.9	22.4			98.5	19.0			97.3	20.3		
	1st	no	91.6	23.2	9.1		89.3	24.8	7.7		88.8	23.7	9.2	
	2nd		100.7	18.7			97.0	21.4			98.0	20.7		
MEF <sub>25</sub> (% pred)	1st	yes	80.8	22.6	11.2		84.1	21.6	12.7*		81.3	22.2	12.3	
	2nd		92.0	24.7			96.8	22.5			93.6	25.0		
	1st	no	90.4	31.4	15.4*		89.7	33.8	8.3		87.7	32.2	12.7	
MEF <sub>25</sub> (% pred)	2nd		105.8	27.3			98.0	29.7			100.4	29.7		
	1st	yes	80.6	29.9	8.9		81.8	26.9	17.8*		81.2	27.0	12.6	
	2nd		89.5	31.8			99.6	31.3			93.8	32.1		

d – difference between the 2nd and the 1st spirometry ; \* Significant increase in lung function between children exposed to smoking or non-smoking parents ( $p < 0.05$ ) ; SD – standard deviation; FEV<sub>1</sub> – forced expiratory volume during the first second; VC – vital capacity; PEF – peak expiratory flow; MEF<sub>75</sub> – mean expiratory flow at 75%; MEF<sub>50</sub> – mean expiratory flow at 50%; MEF<sub>25</sub> – mean expiratory flow at 25%.

**Table 4**  
**Relative risk for a child with asthma to have poor lung function (PLF) before (1st spirometry) and after 6 months (2nd spirometry) of the fluticasone propionate (FP) treatment, if exposed to environmental tobacco smoke (ETS)**

Parameter	Good lung function (GLF)		Poor lung function (PLF)		OR (95% CI)	p
	ETSE	ETSF	ETSE	ETSF		
I Spirometry	FVC (%pred)	252 (74.7)	153 (80.5)	37 (19.5)	1.4 (0.9 to 2.2)	> 0.05
	FEV <sub>1</sub> (%pred)	264 (78.3)	168 (88.4)	22 (11.6)	2.1 (1.3 to 3.5)	< 0.01
	PEF (%pred)	244 (72.4)	159 (83.7)	31 (16.3)	2.0 (1.2 to 3.1)	< 0.01
	MEF <sub>25-75</sub> (%pred)	215 (63.8)	153 (81.0)	37 (19.0)	2.4 (1.6 to 3.7)	< 0.001
II Spirometry	FVC (%pred)	302 (89.6)	186 (97.9)	4 (2.1)	5.4 (1.9 to 15.3)	< 0.001
	FEV <sub>1</sub> (%pred)	301 (89.3)	186 (97.9)	4 (2.1)	5.6 (1.9 to 15.9)	< 0.001
	PEF (%pred)	304 (90.2)	164 (86.3)	26 (13.7)	0.7 (0.4 to 1.2)	> 0.05
	MEF <sub>25-75</sub> (%pred)	263 (78)	177 (93.2)	13 (6.8)	3.8 (2.1 to 7.1)	< 0.001

**FVC – forced vital capacity; FEV<sub>1</sub> – forced expiratory volume during the first second; PEF – peak expiratory flow; MEF<sub>25-75</sub> – mean expiratory flow at 25, 50 and 75% of forced vital capacity; OR – odd ratio; CI – confidence interval; ETSE – ETS exposed children (n = 337); ETF – ETS free children (n = 190); GLF – FEV<sub>1</sub> ≥ 85% pred; PLF – FEV<sub>1</sub> < 85% pred.**

It is very important to educate children with asthma and their parents about the negative effects of the ETS<sup>32,33</sup>. The education may decrease the future ETS exposure of a child. In addition, the smoking habits of parents represent a risk factor associated with the initiation of smoking during adolescence among children<sup>34–36</sup>. Many governments have conducted tobacco control campaigns, however, the adequate implementation of these measures is essential<sup>37</sup>.

The limitation of this study was the investigators' inability to check the real ETS exposure of the children in their home environment. Another limitation was our inability to check parents' active or passive smoking status and passive smoking status of children by measuring carbon monoxide in the exhaled air with Smokerlyzer. Moreover, we did not perform a bronchodilator test with short acting  $\beta_2$ -agonists after the 1st spirometry to assess reversibility of bronchoconstriction in the airways of the ETSF and ETSE children. We thought it would be helpful to assess the lack of reversibility in the ETSE children and would suggest a COPD-like component of their illness, but the majority of the children (82%) had FEV1  $\geq$  85% of predicted and in most of them the positive BDT could not be obtained. Finally, we were not able to assess direct compliance with the IC therapy.

## Conclusion

The ETSE children had lower values of the lung function tests both before and after the FP treatment. The ETSE children were treated with higher doses of FP than the ETSF children. After 6 months of the IC treatment, both the ETSE and ETSF children significantly improved all lung

function test parameters. However, the ETSE children did not reach the spirometric values of the ETSF children, despite receiving the higher IC doses. The improvement of lung function test parameters under the IC treatment was practically the same in the ETSF and ETSE children, but the improvement in the children of smoking mothers was significantly lower than in the children of smoking fathers. The proportion of children improving from the PLF to GLF after 6 months of FP was much higher in the ETSF than in ETSE children.

This study did not provide enough evidence that the ETS exposure may attenuate the beneficial effect of IC on children with asthma, suggesting that timely protection of children with asthma from adverse effect of ETS makes sense, since there is no proof that their airways respond less to IC, which is the case in adult smokers with COPD and asthma.

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## Hybrid treatment of thoracoabdominal aortic aneurysm: case series and review of the literature

### Hibridni tretman aneurizmi torakoabdominalne aorte: serija bolesnika i pregled literature

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#### Abstract

**Background/Aim.** Open thoracoabdominal aortic aneurysm (ThAAAs) repair is a challenging mission. Total endovascular approach is performed at selected institutions in developing countries, however these are not generalizable. Hybrid procedures offer an alternative approach. The aim of this study was to present our results of the ThAAA hybrid treatment and a literature review. **Methods.** This is a retrospective study of all patients that underwent the hybrid ThAAA repair at our institution between January 2011 and January 2018. Hybrid ThAAA repair was done as a two-staged procedure – open visceral debranching followed by stent-graft placement (TEVAR). The following data from eligible studies were extracted and analyzed: first author, publication year, patient sample, 30-day/in-hospital mortality, permanent paraplegia rate, presence of endoleaks and graft patency after the follow-up period, overall survival and mean follow-up period. **Results.** Seven patients underwent the hybrid ThAAA repair at our institution. Neither intraoperative death nor technical failure due to TEVAR was observed. Mean follow-up rate after discharge was 51.71 months [95% confidence interval (CI): 14.67–88.74

months]. At the time of the follow-up, all bypasses were patent and no prosthesis migration was documented. After one year, a case of type Ib endoleak was identified and treated successfully. Twenty-five studies were eligible for the literature review. Primary technical success was 91.69% (95% CI: 85.34–97.24%). Mean percentage of permanent paraplegia was 5.27% (95% CI: 3.55–7.01%). Visceral graft patency during the mean follow-up of 27.54 months (95% CI: 17.41–37.66 months) was 94.5% (95% CI: 92.5–96.5%). Mean pooled percentage rate of overall endoleaks during the follow-up period was 16.72% (95% CI: 11.15–22.29%). Analysis revealed 15.32% (95% CI: 11.04–19.61%) of 30-day/in-hospital mortality and 65.98% (95% CI: 58.15–73.81%) of overall survival after the follow-up period. **Conclusion.** Although thought as less invasive, the hybrid ThAAA repair is still associated with a considerable morbidity and mortality.

#### Key words:

aortic aneurysm, thoracic; blood vessel prosthesis; vascular surgical procedures; endovascular procedures; treatment outcome.

#### Apstrakt

**Uvod/Cilj.** Otvoreni tretman aneurizmi torakoabdominalne aorte (ThAAA) je značajan i težak poduhvat. Totalni endovaskularni tretman je moguć, ali u specijalizovanim institucijama i nije svuda prisutan. Hibridni tretman nudi alternativan pristup u tretmanu ThAAA. Cilj rada bio je prezentovanje naših dosadašnjih rezultata hibridnog tretmana ThAAA, kao i pregled literature. **Metode.** U radu je prikazan restrospektivni pregled svih bolesnika sa hibridnim tretmanom ThAAA u našoj ustanovi u periodu od januara 2011 do januara 2018. Hibridni tretman ThAAA

je učinjen u dve faze: prvi stadijum je podrazumevao otvoreni visceralni debranching, a drugi, nakon toga, plasiranje stent-grafta u odloženoj fazi. Pregledom literature izdvojeni su i analizirani sledeći podaci: ime prvog autora, godina publikacije, broj bolesnika, 30-dnevni/intrahospitalni mortalitet, procenat bolesnika sa permanentom paraplegijom, prisustvo endolika, prohodnost graftova, sveukupno preživljavanje nakon perioda prećenja i središnja vredost praćenja. **Rezultati.** Ukupno sedam bolesnika sa ThAAA je tretirano u našoj ustanovi. Nijedan slučaj intraoperativne smrti, niti nemogućnost plasmana endografa (TEVAR) nije zabeležen. Srednje vreme praćenja bolesnika je bilo 51,71 meseci (95% interval poverenja (IP): 14,67–88,74 meseci).

Na kraju perioda praćenja svi bajpasevi su bili prohodni i nijedan slučaj migracije stent-grafta nije zabeležen. Posle godinu dana kod jednog bolesnika je zabeležen endolik tip Ib koji je uspešno tretiran. Ukupno dvadeset-sedam studija je izdvojeno za literaturni pregled. Tehnički uspeh je bio 91,69% (95% CI: 85,34–97,24%). Srednja vrednost permanentne paraplegije je bila 5,27% (95% CI: 3,55–7,01%). Objedinjena stopa prohodnosti graftova tokom središnjeg perioda praćenja od 27,54 meseci (95% CI: 17,41–37,66 meseci) je bila 94,5% (95% CI: 92,5–96,5%). Objedinjena stopa endolikova tokom perioda praćenja je bila 16,72% (95% CI: 11,15–22,29%). Objedinjena stopa

30-dnevnog/intrahospitalnog mortaliteta je bila 15,32% (95% CI: 11,04–19,61%), a stopa preživljavanja tokom perioda praćenja od 27,54 meseci 65,98% (95% CI: 58,15–73,81%). **Zaključak.** Uprkos manjoj invazivnosti, hibridni pristup za tretman ThAAA je i dalje povezan sa značajnim morbiditetom i mortalitetom.

#### Ključne reči:

**aneurizma, torakalna; krvni sudovi, proteze; hirurgija, vaskularna, procedure; endovaskularne procedure; lečenje, ishod.**

## Introduction

Surgical repair of thoracoabdominal aortic aneurysms (ThAAAs) is a challenging both for a patient and a surgeon. The results of high volume centers are excellent, where the cumulative 30-day mortality is even less than 10% in selected series<sup>1,2</sup>. Overall mortality was shown to be 10% according to the American College of Surgeons National Surgical Quality Improvement Program from 2005 to 2010<sup>3</sup>. Total endovascular approach is available at the selected institutions. This approach is associated with better outcomes, lower rates of mortality, morbidity and spinal cord ischemia<sup>4-6</sup>. Unfortunately, these are not generalizable due to the anatomic restrictions, problems with access to devices and need for custom fabrication as well as their costs.

Hybrid procedures are an alternative approach to the ThAAA management. Because it is a combination of standardized open techniques and off-the-shelf endografts, this is applicable to a wide range of patient anatomy. This approach offers the potential advantage of being less invasive since

they avoid the extensive two cavity exposure, aortic cross-clamping and mechanical circulatory support that comprise open ThAAA repair.

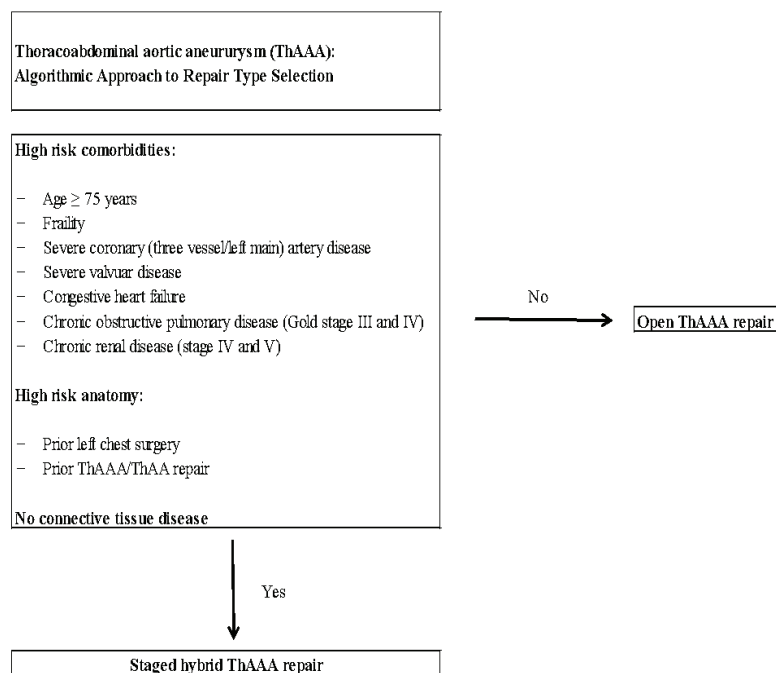
In this paper we present our results of the hybrid procedures for a complex thoracoabdominal aortic aneurysm in seven patients with the ThAAA and a review of the literature.

## Methods

### Patient population

This is a retrospective study of all patients that underwent the hybrid ThAAA repair at our institution between January 2011 and January 2018. A total of seven consecutive patients were identified. The study was approved by the Ethics Committee of our institution and was conducted according to the principles of the Declaration of Helsinki.

At our institution, there is a clear protocol for the ThAAA treatment (Figure 1).



**Fig. 1 – Algorithm for thoracoabdominal aneurysm (ThAAA) repair at our institution.**

Patients aged  $\geq 75$  years, with a severe coronary artery (three vessel/left main) or severe valvular disease, congestive heart failure, chronic obstructive pulmonary disease (Gold class III and IV), chronic kidney disease (stage IV and V), prior left chest surgery, prior open ThAAA/ThAA repair and no connective tissue disease undergo staged hybrid ThAAA repair. There were a total of 174 elective patients with the ThAAA admitted to our institution in period between January 2011 and January 2018. By using this algorithm, 111 patients were treated with open repair, 7, as previously said, with the hybrid approach and 56 patients were rejected due to a high risk of both strategies.

Baseline characteristics of patients are shown in Table 1. The specific pathologies were as follows: five degenerative ThAAA (three type III and two type V) and two chronic post-dissected ThAAA (both type I). All patients underwent a thorough preoperative assessment which included a computed tomography (CT) scan to evaluate the extent of the aortic pathology and to select the endoprosthesis size (Figure 2) and transthoracic echocardiography. Technically, a successful placement of the stent-graft was defined as a successful deployment of the graft without mortality, endoleaks and conversion to open surgery or graft occlusion within the first 24 hours. CT scan was done in all patients before discharge (Figure 3). Follow-up CT scans were performed immediately after the intervention, as well as after the first and sixth month, and after that on a year basis.

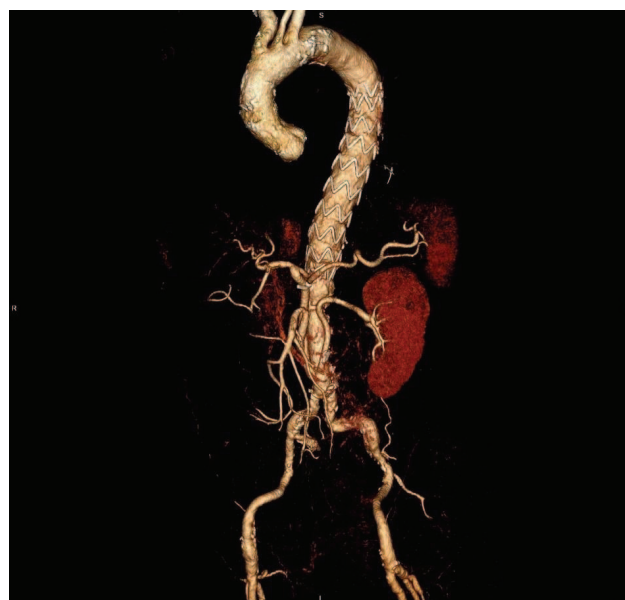


**Fig. 2 – Preoperative computed tomography (CT) of the patient with type III thoracoabdominal aortic aneurysm (ThAAA).**

#### *Technical details*

Hybrid ThAAA repair was done as a two-staged procedure, which consisted of the open visceral debranching and stent-graft placement. The first stage of visceral debranching

was achieved in all cases from a transabdominal approach via midline laparotomy. Prosthetic retrograde bypass grafts (all Dacron prosthesis) were constructed from the common iliac arteries or a Dacron graft which was used for the reconstruction of infrarenal AAA. Visceral target vessels were ligated proximal to the revascularization to prevent endoleak after the endoprosthesis placement. Celiac artery was identified through omentum minus and its revascularisation was performed in all cases with a bypass to the common hepatic artery in order to preserve visceral circulation. The superior mesenteric artery (SMA) was identified in some cases through omental bursa and in other at the base of the transverse colon. First option through the omental bursa has an advantage of being proximal to the origin of the middle colic artery allowing ligation of the SMA just proximal to the graft. The left and right renal artery were identified as they emerge from the aorta with the right renal artery behind the inferior vena cava. The prosthetic bypass grafts were used as a variety of custom-branched graft configurations.



**Fig. 3 – Postoperative computed tomography (CT) of the same patient with type III thoracoabdominal aortic aneurysm (ThAAA).**

The second stage of the hybrid repair consisting of the stent-graft placement was performed in a delayed fashion since we did not have a hybrid operating room. All patients remained hospitalized between the first and second stage. Through the transfemoral approach in all 7 cases, a diagnostic aortography was accomplished with a pigtail catheter. An endoprosthesis was selected in order to cover the entire lesion and was oversized by 10%–20% according to the diameter of landing zones. An overlapping between two stent-grafts or between the stent-grafts and the Dacron prosthesis was done according to the instruction for use protocol for each type of graft. The postoperative mean blood pressure was kept above 100 mmHg and cerebrospinal fluid (CSF) drainage was used for the first 72 hours, as per our institutional protocol for the SCI prevention.

Table 1

## Preoperative characteristics and comorbidities

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Gender	male	male	female	male	male	male	male
Age (years)	66	74	73	75	69	70	67
Hypertension	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Diabetes on insulin	No	No	Yes	No	No	Yes	No
COPD	No	Yes	No	No	No	No	Yes
Smoker	Yes	Yes	No	Yes	Yes	Yes	Yes
Hyperlipidaemia	Yes	No	No	No	No	Yes	Yes
BMI (kg/m <sup>2</sup> )	30.5	23.6	37.5	33.6	28.9	36.3	23
Chronic renal failure	Yes	No	Yes	Yes	No	Yes	No
Ejection fraction < 50%	No	No	Yes	No	No	Yes	No
Connective tissue disease	No	No	No	No	No	No	No
Prior stroke	Yes	Yes	No	No	No	Yes	No
Previous aortic surgery	AA replacement	No	No	AA replacement	No	AVR	No
ASA score	3	3	3	3	3	4	3
Aortic pathology	Postdissectional aneurysm	Degenerative aneurysm	Degenerative aneurysm	Degenerative aneurysm	Degenerative aneurysm	Postdissectional aneurysm	
Max. aortic diameter (cm)	7.2	6.8	7.0	8.4	6.7	9.1	7.5
Symptomatic	No	No	No	No	No	No	No
Status	Elective	Elective	Elective	Elective	Elective	Elective	Elective

**COPD** – chronic obstructive pulmonary disease; **BMI** – body mass index, **AA** – ascending aorta; **AVR** – aortic valve replacement.  
**ASA** – American Society of Anesthesiologists.

### Literature search strategy

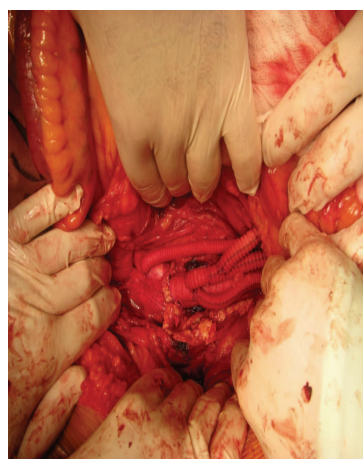
All paper that were published about the hybrid ThAAA treatment until March 2018 were extensively searched. The following medical subject heading term search strategy for identification of the eligible studies was used: “thoracic”, “thoracoabdominal”, “aortic aneurysm”, “endovascular”, “stent-graft”, “visceral bypass graft”, “visceral revascularization”, “visceral debranching”, “hybrid”. The following electronic search engines were used: Google Scholar, Medline, Scopus and Cochrane Library. Furthermore, all retrieved articles were examined for a relevant case series. Hybrid repair should consist of two stages: open debranching followed by the endoprosthesis placement. Both staged and single procedures were analysed. The eligible study for this review should have: 1) a visceral bypass followed by the endoprosthesis placement; 2) at least one basic outcome criteria; 3) series of at least 15 patients. Exclusion criteria were: articles that were not in English, studies with the hybrid aortic arch debranching, case reports and papers with less than 15 patients. Some papers also reported patients with the combined visceral debranching and endovascular exclusion for different thoracoabdominal pathologies. These were also taken into account and analyzed as a part of a greater cohort of patients only if the data for them were separately shown. The eligible studies for this review were independently assessed by two reviewers. Data that were extracted and analyzed were: first author, year of publication, patient sample, 30-day/in-hospital mortality, percentage of permanent paraplegia, presence of endoleaks and graft patency after the follow-up period, overall survival and the mean follow-up period.

### Results

Three patients underwent a concomitant infrarenal AAA repair with the visceral debranching (Figure 4). In other four cases, the visceral debranching was done as a retrograde bypass graft from the common iliac arteries. In one case, the hybrid aortic arch repair was previously performed. Technical success was achieved in 6 (85.71%) cases. Neither intraoperative death nor technical failure due to TEVAR was observed intraoperatively. Six patients were extubated 24 hours after the surgery. One patient was re-intubated on postoperative day 4 (POD 4) and re-intervened because of the peptic ulcer perforation and kept ventilated for 6 days due to a respiratory failure secondary to pneumonia. In one case, there was an early AMS graft occlusion which required early re-intervention and new bypass creation. No postoperative transient or permanent SCI occurred. In all cases the CSF drainage was performed for 72 hours as per protocol. Follow-up rate was 100%. Mean follow-up rate after discharge was 51.71 months (95% CI: 14.67–88.74). After the follow-up period, all bypasses were patent and no case of a stent-graft migration was noted. After one year, a case of type Ib endoleak was documented in patient number 7. This complication was successfully treated by endovascular means later on. Table 2 shows the intra- and postoperative data.

An abstract review showed that 168 studies out of total 539 were considered relevant. Out of 168, 143 papers were

excluded when the inclusion and exclusion criteria were applied. A total of 1,138 patients from 25 studies were analyzed. Pooled primary technical success rate was 91.69% (95% CI: 85.34–97.24%). Mean percentage of permanent paraplegia was 5.27% (95% CI: 3.55–7.01%). The visceral graft patency pooled rate during the mean follow-up period of 27.54 months (95% CI: 17.41–37.66 months) was 94.5% (95% CI: 92.5–96.5%). Mean pooled percentage rate of overall endoleaks during the follow-up period was 16.72% (95% CI: 11.15–22.29%). One hundred and sixty-six patients, 14.58% (95% CI: 11.04–19.61%) died during the first 30 postoperative days. The pooled rate of overall survival after the mean follow-up period of 27.54 months was 65.98% (95% CI: 58.15–73.81%) (Table 3)<sup>7–31</sup>.



**Fig. 4 – Intraoperative photo after the complete visceral debranching with simultaneous abdominal aortic aneurysm (AAA) repair.**

### Discussion

Since the first introduction of this hybrid procedure at our institution<sup>32</sup>, we have successfully treated patients with this approach because they were in a high risk for open ThAAA repair. Visceral debranching can be accomplished via a standard median laparotomy, without entering the chest cavity, and without the aortic cross-clamping or mechanical circulatory support. That is why the hybrid repair is “less-invasive” than the open ThAAA repair and results in less physiological derangement. The real-world data on the other side, show mixed results (Table 3), leaving ambiguity of the exact role of hybrid repairs<sup>7–35</sup>.

This literature review was performed with a purpose to explore contemporary results and concerns of the technique. In order to avoid the bias of the small sample, we excluded case reports and small case series. When analyzing the morbidity and mortality rates, a significant heterogeneity was found, with the mean pooled rate of 14.58% for 30-day mortality and 5.27% for an irreversible spinal cord injury (SCI). Similar results were presented in the Society for Vascular Surgery (SVS) meeting in 2012 from the North American Complex Abdominal Aortic Debranching (NACAAD).

Table 2

Intraoperative and postoperative data						
Intraoperative and postoperative data	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Concomitant AAA repair	Yes	No	No	Yes	Yes	No
ThAAA class	I	III	V	III	III	I
Number of endografts	3	3	3	3	2	3
Endografts	Valiant Captiva®	Bolton Relay®	Zenith TX2®	Valiant Captiva®	Zenith TX2®	Valiant Captiva®
Proximal stentgraft diameter (mm)	28	42	40	42	36	46
Ventilation > 24 hours	No	No	No	No	No	No
Dyalisis	No	No	No	No	No	No
Stroke	No	No	No	No	No	No
SCI	No	No	No	No	No	No
Pneumonia	No	No	No	No	No	No
ICU length of stay (days)	3	5	6	5	5	12
In-hospital mortality	No	No	No	No	No	No
Follow-up (months)	102	86	83	57	12	8
Late endoleaks	No	No	No	No	No	No
Aorta related intervention	No	No	No	No	No	No
AAA – abdominal aortic aneurysm; ThAAA class – thoracoabdominal aortic aneurysm (Crawford classification); SCI – spinal cord ischemia, ICU – intensive care unit; EVAR – endovascular aortic replacement.						
						EVAR

Table 3

## Major published series of hybrid thoracoabdominal aortic aneurysm (ThAAA) repair with more than 15 patients

Author	Year	Patients, n	30-day mortality (%)	Permanent paraplegia (%)	Endoleaks (%)	Graft patency (%)	Overall survival (%)	Mean follow-up (days)
Zhou et al. <sup>7</sup>	2006	31	3.2	0	6	95	90% <sup>a</sup>	16
Black et al. <sup>8</sup>	2006	22	23	0	42	98	-	9.5
Lee et al. <sup>9</sup>	2007	17	24	0	12	96	76 <sup>a</sup>	8
Van der Mortel et al. <sup>10</sup>	2008	16	31	0	13	95	69 <sup>a</sup>	13
Quinones-Baldrich et al. <sup>11</sup>	2009	20	0	6.6	30	100	76 <sup>a</sup>	17
Donas et al. <sup>12</sup>	2009	58	8.6	3.4	17	97	74 <sup>a</sup>	22
Drinkwater et al. <sup>13</sup>	2009	107	15	8.4	33	87 <sup>*</sup>	-	-
Patel et al. <sup>14</sup>	2009	23	17	4	23	90	68 <sup>a</sup>	6
Chiesa et al. <sup>15</sup>	2009	31	19.4	3.2	3.2	93.2	61.3 <sup>a</sup>	11.1
Kabbani et al. <sup>16</sup>	2010	36	8.3	0	39	93	80 <sup>a</sup>	6
Patel et al. <sup>17</sup>	2010	29	3.4	3.4	34	95 <sup>*</sup>	-	-
Kuratani et al. <sup>18</sup>	2010	86	2.3	0	17	99	86 <sup>e</sup>	88
Smith et al. <sup>19</sup>	2011	24	12.5	8.3	12	99	-	12
Hughes et al. <sup>20</sup>	2012	58	9	4	-	95	62 <sup>e</sup>	26
Tshomba et al. <sup>21</sup>	2012	52	14	1.9	7.7	93	77 <sup>h</sup>	24 <sup>**</sup>
Bianchini Massoni et al. <sup>22</sup>	2014	45	24	13	-	79	45 <sup>f</sup>	26
Chiesa et al. <sup>23</sup>	2014	55	12.7	-	-	90.2	73.8 <sup>e</sup>	36
Rosset et al. <sup>24</sup>	2014	76	34.2	11.8	2.63	99.14	64.48 <sup>*</sup>	30
Gkremoutis et al. <sup>25</sup>	2014	30	26.7	10	3	95.3	57.8 <sup>b</sup>	12
Benrashid et al. <sup>26</sup>	2016	81	9.9	7.4	12.3	-	32 <sup>e</sup>	60
Jain et al. <sup>27</sup>	2016	19	0	5.26	15.78	100	100 <sup>e</sup>	36
Hawkins et al. <sup>28</sup>	2017	25	4	8	-	-	-	-
van de Graaf et al. <sup>29</sup>	2017	15	33	7	-	-	40 <sup>b</sup>	12
Tsilimparis et al. <sup>30</sup>	2018	22	15.3	4.3	-	92.05	55 <sup>a</sup>	16.4
Shuto et al. <sup>31</sup>	2018	60	5	3	10	95	43.5 <sup>g</sup>	96

\*30 day; \*\*median; a – at end of follow period; b – 1-year Kaplan-Meier survival; c – 3-year Kaplan-Meier survival; d – 4-year Kaplan-Meier survival; e – 5-year Kaplan-Meier survival; f – 6-year Kaplan-Meier survival; g – 8-year Kaplan-Meier survival; h – freedom from aortic-related deaths at end of follow-up.

Their data for 30-day mortality was 16% and for SCI 14%, which was associated with the extent of the aortic disease and aneurysm rupture<sup>36</sup>. Better results in our material might be explained by a low number of patients and careful selection. Available open repair option provides unbiased selection while most patients in our group had the chronic obstructive pulmonary disease (COPD) and obesity as a risk factor with the average age lower than reported.

Substantial mortality associated with this hybrid approach could be explained by the fact that a majority of these patients are elderly and with major comorbidities that cannot withstand this kind of reconstruction. This highlights the importance of patient selection. In our sample of patients, the mean age was 70.57 years, they were mainly obese (71.42%) and all of them had the American Society of Anesthesiologists (ASA) score  $\geq 3$ . In examining the specifics of the patients, as seen from the literature<sup>7-36</sup>, it appears that they tend to be in a higher risk. It seems that hybrid repair can offer some advantage in high-risk patients, but it is certainly not the low-risk approach. Also, even more important is that it demonstrates that there are limits of how far the hybrid technique can be pushed. In the end, in the population of patients with the extremely high-risk, a non-operative approach may be actually the best option.

What is even more interesting point is that this technique was adopted by centers with little previous experience in treating patients with the ThAAA. A few large-volume centers both in the USA and Europe display the constant evidence of better results demonstrating how extensive experience in the treatment of this pathology, learning curve and an organized perioperative background are required for the best management.

There is no significant amount of data which demonstrates whether the endoprosthesis placement for the exclusion of the ThAAA should be done concomitantly or in a delayed fashion<sup>8</sup>. Those who are opponents of staged approach say that this leaves a patient to a potential rupture, although there are little data to support this approach<sup>9</sup>. This approach is associated with longer hospital stay at the Intensive Care Units (ICU) and higher morbidity rate<sup>38</sup>. On the other side, the delayed approach has one major advantage. It allows the patients to recover from debranching surgery, before exposing them to the placement of the aortic endoprosthesis. This has the larger influence on the kidney function, as it allows them to recover from ischemia due to debranching surgery before exposing them to the contrast during the second, endovascular part of the hybrid repair that also has nephrotoxic effect. That is why this double-staged procedure should be mainstay of treatment for the patients who require the extensive ThAAA reconstruction, with single-staged reserved only to those who are at high-risk of aneurysm rupture.

Another concerns are the endograft complications, and on the other side, visceral graft patency. Our patient sample revealed 100% of the visceral graft patency during the mean follow-up of 51.71 months. One (14.28%) patient had an endoleak type Ib treated with additional endograft in the distal landing zone. The literature review data revealed the visceral graft patency of 94.5% during the mean follow-up of 27.54 months, whereas 16.72% had an endoleak. Kuratani et al.<sup>18</sup>, with the longest follow-up period (88.5 months,  $n = 86$ ), reported two intraprocedural deaths, one patient died because of a visceral graft occlusion and bowel necrosis and the other because of a graft infection.

Due to financial and technical reasons we do not perform branched or fenestrated procedures in our country. Inaccessibility of these treatment options brings non-selectivity, but on the other hand, our institution has extensive experience with the open repair<sup>37-41</sup> as an option in treatment of this complex pathology. For unfit patients, the hybrid repair is the only viable option. We think that a thorough case selection and a choice of the right procedure are two most important things. In large-volume centers or those with suitable expertise, visceral hybrid repair is an alternative treatment strategy for the high-risk population of patients with the limited open and fully endovascular therapeutic alternatives.

Our study has several limitations. Firstly, this was a retrospective study with prospectively collected data in a single center where a patient accrual required nearly seven years. Secondly, our study was performed on a small number of patients. Thirdly, there was an absence of a control group. The main strength of this study is detailed clinical information, especially in the literature review which provides enough statistical power to make valid conclusions.

## Conclusion

Although thought as less invasive, hybrid ThAAA repair is still associated with a considerable morbidity and mortality rate since it is frequently performed in fragile patients. Despite the improvements in the branched and fenestrated procedures, hybrid approach may continue to have a role in the hostile anatomy of those patients or when advanced stent grafts are not available. That is why this should be reserved for the large-volume centers with enough experience that can determine a patient suitability and offer all treatment options with high standards of perioperative care.

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## Large hibernoma of the neck: A case report

### Veliki hibernom vrata

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#### Abstract

**Introduction.** A hibernoma is a rare benign tumor derived from vestigial remnants of brown adipose tissue. In neonates this tissue makes up about 5% of the body mass and its amount greatly decreases after birth, persisting only in scattered subcutaneous areas. In rare cases, brown fat continues to grow leading to a hibernoma that may be located in the head and neck. We present an illustrative case of a large hibernoma of the neck with infraclavicular extension and discuss about diagnostic and treatment difficulties. **Case report.** A 29-year-old male presented with large, slowly progressive, painless neck mass that was noticed 6 months earlier. Computed tomography (CT) and magnetic resonance (MR) showed a well-vascularized, soft tissue tumor of the lateral region of the neck and supraclavicular fossa with extension below clavicle. Treatment included arterial embolization followed by challenging surgical removal of the tumor. Dissection was performed at III, IV and V levels of the neck, making complete resection possible without the tumor fragmentation or major blood vessels and cranial nerves injuries. The final diagnosis of the hibernoma was made by histopathological analysis. The patient had no signs of recurrence during three-year follow-up. **Conclusion.** Although the CT scan and MR may raise the suspicion, hibernoma is definitely diagnosed by a pathologist. It is very important to exclude the malignant processes, foremost liposarcoma. The tumor fragmentation during surgery should be avoided because the high vascularity of the tumor tissue carries a substantial risk for hemorrhage. Our experience with preoperative embolization and complete tumor resection in this case showed positive impact on the final outcome.

#### Key words:

diagnosis, differential; embolization, therapeutic; head and neck neoplasms; lipoma; male; otorhinolaryngologic surgical procedures; tomography, x-ray computed.

#### Apstrakt

**Uvod.** Hibernom je redak benigni tumor poreklom od zaostataka mrkog masnog tkiva. Kod novorođenčadi ovo tkivo čini oko 5% telesne mase i njegova zastupljenost prilično opada nakon rođenja, zaostajući samo u rastrkanim potkožnim regionima. U retkim slučajevima, mrko masno tkivo nastavlja da raste, formirajući hibernom koji se može naći u regiji glave i vrata. Prikazujemo ilustrativan slučaj velikog hibernoma vrata sa širenjem ispod nivoa klavikule i diskutujemo o dijagnostičkim i terapijskim smernicama. **Prikaz bolesnika.** Na otorinolaringološki pregled se javio 29-godišnji muškarac zbog velikog, spororastućeg, bezbolnog izraštaja na vratu, koji je primetio šest meseci ranije. Kompjuterizovana tomografija (CT) i magnetna rezonanca (MR) pokazali su dobro vaskularizovan, mekotkivni tumor bočne strane vrata i nadključne jame sa širenjem ispod nivoa ključne kosti. Lečenje se sastojalo od arterijske embolizacije tumora, praćene izazovnim hirurškim uklanjanjem tumora. Disekcija je obuhvatila III, IV i V nivo vrata, omogućivši kompletnu resekciju bez fragmentiranja tumora, kao i bez povreda velikih krvnih sudova ili kranijalnih nerava. Konačna dijagnoza hibernoma postavljena je patohistološkom analizom. Tokom trogodišnjeg praćenja bolesnika nije bilo znakova ponovnog nastanka bolesti. **Zaključak.** Iako CT i MR mogu da postave sumnju na hibernom, definitivnu dijagnozu postavlja patolog. Veoma je važno isključiti postojanje maligniteta, najpre liposarkoma. Trebalo bi izbegavati fragmentaciju tumora tokom operacije zato što izrazita vaskularizacija tumora nosi sa sobom rizik od krvarenja. Naše iskustvo sa preoperativnom embolizacijom i kompletnom resekcijom tumora u ovom slučaju je imalo odličan rezultat kao konačni ishod lečenja.

#### Ključne reči:

dijagnoza, diferencijalna; embolizacija, terapijska; glava i vrat, neoplazme; lipom; muškarci; hirurgija, otorinolaringološka, procedure; tomografija, kompjuterizovana, rendgenska.

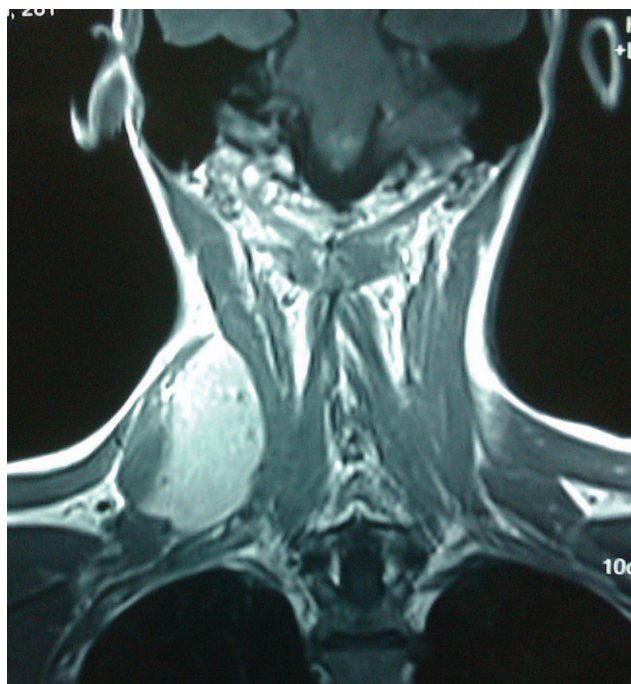
## Introduction

A hibernoma is a rare benign tumor derived from the vestigial remnants of brown adipose tissue. In neonates, this tissue makes up about 5% of the body mass, acting as a kind of a thermal regulator with a high potential to generate heat<sup>1</sup>. Over the first few years, it undergoes rapid degradation, persisting in adults in scattered subcutaneous areas, mostly in the interscapular part of the back<sup>2</sup>. The small depots of brown fat may persist in supraclavicular area of the neck and in rare cases it continues to grow, leading to a benign soft tissue tumor – a hibernoma.

We present an illustrative case of a large hibernoma of the neck with infraclavicular extension and discuss about diagnostic and treatment difficulties.

## Case report

A 29-year-old male patient presented with a large, slowly-progressive, painless neck mass that was noticed 6 months earlier. The patient had no neurological deficits and had no problem of swallowing and breathing. A clinical examination showed a soft-tissue tumor, localized in the lower and lateral parts of the neck on the right side, fulfilling the supraclavicular fossa. There was no pathological process found in the pharynx, larynx or nasal cavity. Computed tomography (CT) and magnetic resonance (MR) showed a well-vascularized, soft tissue tumor of the lateral region of the neck and supraclavicular fossa with extension below clavicle that measured  $14 \times 7 \times 4$  cm (Figure 1). A few reactive lymph nodes measuring less than 1 cm in the short axis diameter were found close to the internal jugular vein during the ultrasound examination of the neck.



**Fig. 1 – Computed tomography (CT) scan of a large neck hibernoma – coronal view.**

Pathological vascularization mostly originated from the deep cervical branches of subclavian artery found on the angiographic examination was reduced by selective embolization (Figure 2). In order to make an accurate diagnosis, fine-needle aspiration cytology was performed. Unfortunately, the obtained material was insufficient to make a definite conclusion.



**Fig. 2 – Angiographic examination of the right vertebral, subclavian and external carotid arteries.**

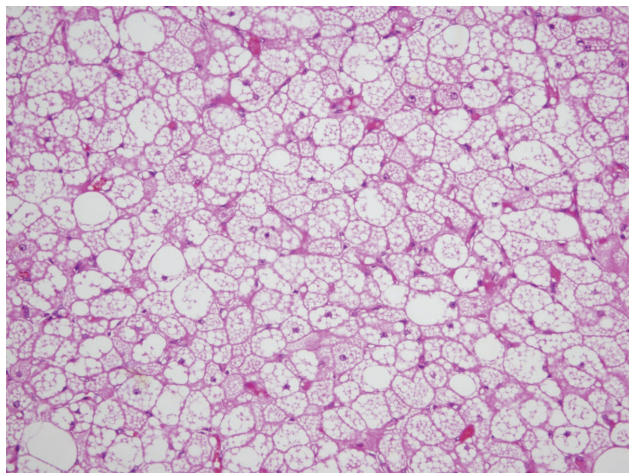
Embolization was followed by a challenging surgical removal of the tumor. Dissection was performed at III, IV and V levels of the neck, making complete resection possible without the tumor fragmentation, or major blood vessels and cranial nerves injuries (Figure 3).



**Fig. 3 – Large hibernoma of the neck after complete surgical resection.**

A final diagnosis of the hibernoma was made by the histopathological analysis after the surgical resection. The tumor histologically resembles brown fat, consisting of the oval cells with the small centrally placed nuclei without pleomorphism and multivacuolated cytoplasm (Figure 4).

The patient had no signs of recurrence during the three-year follow up.



**Fig. 4 – Histological appearance of hibernoma**  
[hematoxylin-eosin (HE), ×200].

### Discussion

Recent studies using the fluorodeoxyglucose positron emission tomography scanning (PET/CT) have shown that brown fat remains in very small amounts in adults, which makes a hibernoma, a very rare benign tumor<sup>3</sup>. There are about 15 reports in literature concerning hibernoma of the head and neck which is not as usual area as thigh, shoulder or back. Although it is commonly found in adults, there is a report of the multiple hibernomas in a 1-month-old infant<sup>4</sup>. Data concerning the gender predominance are contradictory, although there is a justified opinion that hibernomas are more frequent in the male population<sup>5</sup>.

A hibernoma of the neck usually presents as a slowly-progressive, nontender, mobile mass of a different size, ranging from 5 to 10 cm. Silent growth is the characteristic that makes it diagnosed as a large outgrowth in the soft tissue with no specific symptoms. It was reported that the average period from occurrence to diagnosis of a hibernoma is about 2.5 years<sup>5</sup>. Although some abdominal hibernomas demand an immediate surgical procedure<sup>6</sup>, there are no emergency cases of the head and neck hibernoma found in literature.

Considering an important role in nonshivering thermogenesis and high energy demands, the brown fat tissue is made of highly vascularized and mitochondria-rich cells. Therefore, a surgery of the hibernoma is usually associated with intensive intraoperative bleeding. On the other hand, this increased vascularity may help to differentiate hibernomas from other soft-tissue tumors. Some authors recommend arterial embolization to be carried out before the surgery in order to minimize the bleeding that may occur during the intervention<sup>7</sup>. There are no literature evidence that preoperative embolization of a hibernoma affects the surgical outcome, although it may help the surgeon to perform complete resection without the tumor fragmentation<sup>8</sup>.

A hibernoma is typically seen on the CT scan as a low-attenuation mass with linear septations. After the contrast administration, enhancement is clearly seen within the septae and more diffusely within the tumor. On a T1- and T2-weighted

MR imaging, a hibernoma demonstrates a signal intensity that is increased or approximate to the subcutaneous fat tissue<sup>9</sup>. An increased T1-weighted signal intensity may be seen also in the imaging of other benign or malignant mesenchymal tumors and specificity is not high enough to make an accurate diagnosis. Diagnostic considerations should include numerous neoplastic lesions, such as lipoma, angiolipoma, hemangioma, hemangiopericytoma, hemangioblastoma, liposarcoma or some types of sarcoma. However, the MR angiography finding of a high T1 signal intensity, combined with a large, curvilinear branching vessels should raise a suspicion of a hibernoma and narrows the differential diagnosis<sup>10</sup>.

In suspected cases of a hibernoma with inconclusive radiological findings, a fine-needle aspiration cytology is recommended. A core needle biopsy carries a risk of excessive hemorrhage due to hypervascularity of the tumor and should be avoided, although there are numerous reports considering series of patients with hibernoma that underwent percutaneous biopsy without any complications<sup>11–14</sup>.

In cases of the inconclusive cytological findings, the definite diagnosis is made by the postoperative histopathological analysis. There are four histologic types of hibernomas: lobular, myxoid, lipoma-like and spindle cell type of hibernoma. The lobular type is the most common variant of the hibernoma found in 82.4% of the cases<sup>15</sup>, including our patient. Oil red O staining is used for the identification of a lipid component and S100 protein positive neoplastic cells are found on immunohistochemistry in about 85% of hibernoma cases<sup>15</sup>.

According to their clinical, radiological and pathological characteristics, hibernomas are benign tumors with no potential to cause a fatal outcome. A case report from 1967 by Lowry and Halmos<sup>16</sup> suggested a possible malignant nature of hibernoma due to muscular invasion, although authors had a suspicion about the definite diagnosis and considered a liposarcoma as more accurate pathology finding. In another case of the neck hibernoma, focal infiltration of scalene muscles was not considered as a sign of malignancy, but muscular adherence by the benign tumor?<sup>13</sup>.

Furlong et al.<sup>5</sup> reviewed 170 cases of hibernoma and during the follow-up found no metastatic developments or recurrence after complete surgical resection.

Complete surgical resection is considered to be the standard treatment of a hibernoma. Surgeons are recommended to make their best effort to avoid the tumor fragmentation in order to prevent recurrence and minimize the intraoperative hemorrhage. Complete surgical resection provides a good reason to believe that tumor will not reappear, although a long-term follow-up is recommended.

### Conclusion

We find this case interesting because it represents an illustrative additional case to a series of rare tumors published so far in literature. The adequate diagnostic and therapeutic approach made a success in treating this massive neck benign tumor. Our experience with arterial embolization followed by complete surgical resection in this case resulted in a complete recovery of the patient.

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## CASE REPORT



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## Bullous *aplasia cutis congenita* – a report of two cases and brief review of the literature

### Bulozna kongenitalna aplazija kože – prikaz dva bolesnika i kratak pregled literature

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#### Abstract

**Introduction.** *Aplasia cutis congenita* (ACC) is a rare condition characterized by the focal absence of skin, and sometimes other underlying structures at birth. It may occur as an isolated defect or associated with other anomalies and defects. Bullous ACC (BACC) is a clinical subtype of the condition with few cases reported in the literature. It presents as a bullous lesion at birth which gradually transforms into an atrophic scar covered by a thin epithelial membrane. It is considered as cutaneous sign of possible neural tube dysraphism. Some cases present with a dark hair around the lesion (the hair collar sign), which can be even more indicative of neural tube defect. However, cases of BACC reported till today are inconclusive regarding this connection. **Case report.** We report a two cases of BACC of the scalp, in one patient associated with hair collar sign without neural tube defects and the other with hemangioma and we give a brief review of the selected literature. **Conclusion.** Bullous or membranous *aplasia cutis congenita* is benign condition, but may represent as a cutaneous marker of occult neural tube defect. Recognising the condition is important in order to rule out associated anomalies.

#### Key words:

congenital abnormalities; ectodermal dysplasia; scalp; hypertrichosis; hemangioma.

#### Apstrakt

**Uvod.** Kongenitalna aplazija kože je retko oboljenje, koje predstavlja lokalizovan nedostatak kože, a nekada i dublje smeštenih tkiva, prisutan na rođenju. Javlja se kao izolovani poremećaj ili udružen sa drugim anomalijama. Bulozna *aplasia cutis congenita* – ACC (BACC) je klinički podtip kongenitalne aplazije kože o kome ima malo objavljenih slučajeva. Manifestuje se buloznim lezijama prisutnim na rođenju, koje postepeno epitelizuju, uz razvoj atrofičnih, ožiljačnih promena. Smatra se kožnim znakom mogućeg disrafizma nervne cevi. Kod nekih bolesnika se oko lezija može videti pramen kose koji je pigmentovaniji od okolne dlake (znak „ogrlice od dlake”) i može još više upućivati na defekt u razvoju nervne cevi. Međutim, u slučajevima koji su do sada objavljeni, ova povezanost nije sa sigurnošću utvrđena. **Prikaz bolesnika.** Prikazujemo dva slučaja BACC kapilicijuma, jedan udružen sa znakom „ogrlice od dlake” bez defekta nervne cevi i drugi sa hemangiomom, uz kratak pregled izabrane literature. **Zaključak.** Bulozna ili mebranozna kongenitalna aplazija kože je benigno stanje koje može predstavljati kožni znak blagog oblika defekta nervne cevi. Prepoznavanje ovog poremećaja je važno kako bi se dijagnostikovalе moguće udružene anomalije.

#### Ključne reči:

anomalije; ektodermalna displazija; skalp; hipertrichoza; hemangiom.

#### Introduction

*Aplasia cutis congenita* (ACC) is a rare condition characterized by the localized absence of the skin, accompanied sometimes with absence of other underlying structures. It

may be isolated defect, or can be associated with accompanied anomalies of the skin or nervous system as well as genetic diseases or syndromes <sup>1, 2</sup>. Bullous ACC (BACC) is a subtype of ACC. It is present at birth in the form of one or several blisters, or with round areas of eroded skin if blisters

already ruptured during delivery. It is rarely seen by dermatologist in its initial bullous form since it appears at birth. Usually the child with BACC is referred to dermatologists with a flat scar once the bullae have already reabsorbed. In this stage, the condition is known as a membranous *aplasia cutis congenita* (MACC). BACC is considered as one of the cutaneous signs of occult neural tube dysraphism. A ring of dark long hairs encircling a congenital skin lesion is referred as hair collar sign. It represents even more convincing neural tube defect marker, considering that it is often, but not always, associated with encephaloceles, meningoceles, and heterotopic brain tissue<sup>3</sup>. Certainly, this association imposes mandatory search for possible comorbidities, and its early recognition is important. Diagnosis is based on clinical findings, and histopathological confirmation is rarely needed<sup>2</sup>.

We present two cases of BACC of the scalp. One case was accompanied by hair collar sign, and the other by hemangioma on the lower back.

### Case report

#### Case 1

A 14-days-old male infant presented to our dermatology department with bullous lesions localized on right parietal scalp. The six bullous lesions were oval, lined one after another, ranging from 5–15 mm in size of longest diameter, filled with clear content. Around described skin area, a collar of dense black hairs, which were thicker than surrounding hair, were present (Figure 1). These skin lesions were noticed at birth. There were no underlying skull defects.



**Fig.1 – Bullous lesion with a rim of hairs on the right parietal scalp of the neonate (the Case 1).**

The infant was born from a non-consanguineous marriage, at term, by spontaneous delivery. There was no history of maternal chicken pox or herpes simplex infection during pregnancy. During pregnancy, his mother was taking beta-blocker (metoprolol) due to gestational hypertension, advised by her obstetrician. Otherwise, the pregnancy was uneventful. The infant was eutrophic, healthy, with neither major nor minor malformations revealed on several physical examinations. Laboratory findings regarding infections, including testing of titer of immunoglobulin M on toxoplasmosis, rubella, cytomegalovirus, and herpes infections, were within normal ranges. Lesion swab was sent for culture and sensitivity, but no pathogens were isolated. Karyotype was normal male. The sonographic exams of brain, abdominal organs and

lumbar and sacral region as well as the ophthalmological exam were unremarkable.

The diagnosis of BACC was set, and local therapy, including gentle cleansing followed by gentamicin ointment, was recommended. The bullous lesions drained spontaneously and a flat atrophic scar was formed during the first few months of life. The infant was followed-up by a dermatologist as well as a neonatologist. At the age of 9 month, all the lesions were of the approximately same size and shape as at birth, but with the residual atrophic scarring instead of bullae. Hair collar sign was still visible. Motor and mental development of the infant was normal.

#### Case 2

A 7-month-old girl was presented with a four round, slightly erythematous atrophic areas of alopecia, varying in size from 5 to 17 mm in diameter, partially covered with a thin crustose membrane. The lesions were localized at the scalp, near the vertex (Figure 2A).



**Fig. 2 – Clinical manifestations of bullous *aplasia cutis congenita* (BACC) in the 2nd presented patient (the Case 2).**

**A) A round, membranous bald macule, with crustose membrane near the vertex of the scalp; B) Membranous bald macule near the vertex of the scalp and hemangioma on the lower middle part of the back.**

Underlying bone was not affected. At the lower middle part of the back, above vertebra, infantile hemangioma was present. It was in a form of irregular rectangle, less than 1 mm above surrounding skin, 35 x 12 mm in size (Figure 2B). At the left gluteus, near the intergluteal cleft, oval pigmented nevus of about 5 mm in diameter was present. The lesions on the scalp were present at birth. They were described as two oval lesions of about 10 mm in size and two of about 3–4 mm. The lesions imposed as areas of bare dermis, with red and moist surface and slightly raised edges. On one of the bigger lesions bulla was still present and the others seemed as if the bullae had already ruptured. Infantile hemangioma appeared at the age of about 2 weeks, and was smaller at first, but enlarged slowly.

The infant was born as the first child from non-consanguineous marriage. Pregnancy as well as the delivery was completely uneventful. At birth, the baby was eutrophic, healthy, with no visible major and minor congenital anomalies. The laboratory findings regarding infections and sonographic exam of the brain and abdominal organs were unremarkable.

Right after birth, on the scalp lesions just mild local antiseptic was applied. At the age of 2 weeks crusts formed and persisted for several months.

At the age of 7 months, when the infant was referred to us for the first time, the crust was still present; we recommended gentamicin ointment for BACC and the local therapy with timolol-maleate 0.5% gel for hemangioma. Two months later scalp lesions were in the form of oval bald areas of atrophic skin, slightly pinkish, with no crust. Hemangioma was of the same size and shape, but almost flat and with initial signs of regression in the middle. The local therapy was regularly applied. Nevus was unchanged.

## Discussion

ACC is a heterogenous group of disorders characterized by the absence of skin in a localized or widespread area at birth. It is a rare condition, with the estimated incidence of 3 in 10,000 births<sup>1,2</sup>. ACC manifests as a solitary defect of the scalp in 70% of cases, but sometimes it may occur as multiple lesions, or can be found on trunk or extremities. ACC is most often a benign isolated defect, but it can be associated with other physical anomalies or malformation syndromes. It can be accompanied with absence of underlying structures such as bone or dura (20–30% of patients). There are described cases of association with hydrocephaly, linear epidermal nevus, hemangiomas, and multiple defects<sup>1–5</sup>. BACC or MACC is a clinical subtype of this condition, with extremely few cases reported in the literature, either because of underreporting or due to a rare occurrence<sup>1,4–7</sup>. The lesions are cystic or bullous at birth which transform over time into an atrophic, flat scar covered by a thin epithelium. Some authors equate the terms “bullous” and “membranous” in the description of this subtype of lesion<sup>1,3</sup>.

In 1960, O'Brien and Drake<sup>4</sup> reported 5 patients with BACC on the scalp, of which in one female infant 0.5 cm hemangioma of the left upper arm was also present. In 1993,

Fryburg and Greer<sup>5</sup> reported an infant with BACC above the back hairline accompanied by extensive unilateral linear epidermal nevus and 2 small hemangiomas on the upper portion of the back. In both cases of BACC accompanied by hemangiomas no other congenital anomalies were found, the same as in our Case 2.

Sometimes BACC is accompanied by a collarette of dark, coarse hair around the skin defect, so called hair collar sign<sup>3,8,9</sup>. Only few cases of BACC with the hair collar sign have been previously reported<sup>3,7</sup>. In 1995, Drolet et al.<sup>3</sup> reported 6 patients with MACC. Two of the patients had single lesions, the rest had multiple lesions. In all cases a hair collar sign was present. Imaging studies failed to identify intracranial anomalies. Of the 6 reported patients, the bone defects and the associated findings (port-wine stain, meningeal arteriovenous fistula and corneal change) were diagnosed in 3. These patients were not reported to have any other major abnormal physical findings. In 2005, Fujita et al.<sup>7</sup> reported two cases of MACC surrounded by a rim of hairs, one case associated with dense dermal melanocytosis and the other with *naevus flammeus*, but no other anomalies or defects. The present Case 1 of BACC and hair collar sign was without neural tube defects and associated malformations, as well.

Drolet et al.<sup>3</sup> proposed that MACC is a form fruste of a neural tube defect. The patients had a hair collar sign that is regarded as a relatively specific marker for cranial neural tube closure defects. In 2003, Colon-Fontanez et al.<sup>1</sup> presented a new case of BACC and summarize the clinical and histologic findings of the 16 cases reported previously. Some, but not all of the cases were presented with a hair collar sign, too. The histological appearance was similar in all patients evaluated, a distinct pattern containing fibrovascular stromas, edematous stroma, or both. The identical histologic findings can be found in encephaloceles and meningoceles. Finally, the membranous posterior cranial closure sites in the neural tube defects resemble the findings in MACC. This supports a hypothesis that BACC may represent the forme fruste of a neural tube closure defect<sup>1,10</sup>.

The etiology of ACC is unknown. It is probably a combination of genetic factors, maternal medications, compromised vasculature to the skin, infection, and intrauterine trauma<sup>2,11,12</sup>. The configuration, distribution, and clinical appearance of BACC would suggest incomplete closure of embryonic fusion lines, rather than vascular interruption or trauma to the skin<sup>13</sup>. Most reports of BCC are sporadic, however because some forms of ACC are inherited, genetic counseling of the family regarding the risk of recurrence is recommended<sup>2</sup>. In all patients with ACC, a complete obstetric and family history should be obtained. History should include a review of maternal medications (eg, methimazole, carbimazole, misoprostol, valproic acid) and infections such as varicella or herpes simplex viruses during the pregnancy<sup>2,12,14</sup>. Metta et al.<sup>15</sup> reported BACC in an infant with HIV association. ACC was described in infants exposed *in utero* to antithyroid drugs and valproic acid, but it was not related to metoprolol<sup>12,14</sup>. BACC and metoprolol intake during pregnancy in our Case 1 may be a random association and similar observations are needed to suggest causality.

The differential diagnosis of bullae present at birth should include infections and hereditary disorders with increased skin fragility<sup>16</sup>. Key toward the BACC diagnosis is existence of lesions at the very birth, on a limited, localized area of the skin, good general health of the newborn and excluding laboratory findings. But possibility of accompanied congenital anomalies must be ruled out.

The course of BACC depends to some degree on the size of the defect. The lesion almost always heals spontaneously, assuming that underlying structures are not affected. If the defect is small, which is most often the case, recovery is uneventful, with gradual epithelialization and formation of a hairless, atrophic scar over several weeks. Management remains controversial, guided by location, size, and depth of the defect. Because of frequent coloniza-

tion and possible superinfection of existing bullae and erosions, conservative treatment is chosen (silver sulfadiazine, antibiotic ointments or petrolatum) in most of the cases. Larger defects that extend into the dura or cover large skin area, need early surgery<sup>17, 18</sup>.

### Conclusion

BACC is an extremely rare or much underreported type of ACC, of still unclear etiology. It can rarely manifest as multiple bullous lesions on the scalp, as in our patients. Many reports of BACC have no other associated anomalies as in our presented cases. But literature data of accompanied anomalies and syndromes imposes the obligation to rule out such a possibility in every single case of BACC.

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## Ten years of consumption of anti-glaucoma medicaments in Serbia

### Deset godina potrošnje lekova u terapiji glaukoma u Srbiji

To the Editor:

Glaucoma is a chronic disease with irreversible damage of optic nerve<sup>1</sup>. It is the second leading cause of blindness worldwide, and is one of leading causes of preventable blindness<sup>2</sup>. It is estimated that by 2020, about 79.6 million people in world will have glaucoma, and more than 11 million will be consequently bilaterally blind. Prevalence of blindness in all types of disease has been estimated at 5.2 million with 3 million cases with open-angle primary glaucoma<sup>3</sup>. This is predicted to increase substantially as a result of an ageing population and better detection in the community. The recommendations of good clinical practice guidelines for the treatment of glaucoma in the UK are that all cognitive and physical disorders, as well as the existence of relevant comorbidities or potential drug interactions should be taken into account before commencing treatment<sup>4</sup>. Glaucoma disease is a complex, significant public health and socio-medical problem<sup>5, 6</sup>. Glaucoma screening/diagnostics requires a detailed clinical examination of optic nerve and functional analysis/evaluation of patients' field of vision<sup>7, 8</sup>. Early treatment of glaucoma reduces risk of progressive damage to the vision field. Medication therapy is currently the most commonly used as an initial reduction measure for increased intraocular pressure (IOP). The recommendation of the American Academy of Ophthalmology is to consider the relationship between therapeutic effects and adverse drug reactions when selecting anti-glaucoma drugs in order to achieve the desired reduction of increased IOP for each patient<sup>9</sup>. The initial anti-glaucoma therapy includes medications which are individually prescribed and controlled (prostaglandin analogues, beta-blockers, adrenomimetic, parasymphathomimetic, carbonic anhydrase inhibitors)<sup>10-12</sup>.

Today, it is important to monitor the consumption of anti-glaucoma medicaments, because in this way it is possible to estimate the increase of the number of patients and the rise of their awareness that glaucoma must be treated in order to maintain a good quality of life. Thus, it is expected to have an increase in the consumption of these drugs each year.

Our analysis of the consumption of medicaments in treatment of glaucoma in Serbia during eleven-year period (2006–

2016) was made based of the publication "Sale and consumption of drugs in human medicine", published annually by the Agency for Medicinal Products and Medical Devices of the Republic of Serbia (available on Agency's website). The data on drug consumption in glaucoma therapy were analyzed according to the methodology of World Health Organization (WHO) which uses the defined daily dose (DDD) method for this purpose. DDD is a statistical monitoring unit that expresses an agreed amount of a drug according to International Nonproprietary Name (INN), which is most commonly used for the most common indication and is independent of protected name of drug and manufacturer, price, pharmaceutical form and size of package. In general population, the number of DDDs per 1,000 inhabitants per day (DDD/1,000 inhabitants/day) is used. This consumption data based on DDD/1,000 inhabitants/day was obtained firstly by the total consumption of the given drug according to its INN that was expressed in weight or volume units according to the Anatomical-Therapeutic-Chemical Classification (ATC) drug code. All the drugs used in the treatment of glaucoma were then divided into five subgroups: S01EA – sympathomimetics, S01EB – parasymphathomimetics, S01EC – carbonic anhydrase inhibitors, S01ED – beta blocking agents, and S01EE – prostaglandin analogues.

The consumption of the drugs used in glaucoma treatment in Serbia in the period observed is given in Table 1. The highest total consumption of drugs used in the treatment of glaucoma according to the above mentioned methodology was registered in the S01ED group (beta-blocking agents), followed by the S01EE group (prostaglandin analogues) while the other three groups had significantly lower consumption. However, the consumption of all groups had variation in the period observed. For example, consumption of beta-blocking drugs was the highest in 2007, and the smallest one in 2010, but after that an increase was evident. Unequal consumption was also recorded in the group of prostaglandin analogues - by 2011, their consumption grew from year to year, and then in 2012 there was a certain decline. In 2013, there was a rebound of the consumption that was the highest in observed period, but in 2014 a decline in consumption of these drugs was recorded. However, from 2015, trend of an increase in the consumption of prostaglandin analogues was again recorded.

**Table 1**

**Medicaments in the glaucoma treatment, DDD/1,000 inhabitants/day in Serbia in the period 2006–2016**

Year	ATC groups				
	S01EA (sympathomimetics)	S01EB (parasympathomimetics)	S01EC (carbonic anhydrase)	S01ED (beta- blocking agents)	S01EE (prostaglandin analogues)
2006	0.01	1.05	0.32	6.22	0.64
2007	0.05	0.35	0.30	7.89	0.93
2008	0.16	0.66	0.53	7.12	1.48
2009	0.21	0.39	0.48	5.23	2.28
2010	0.45	0.57	0.46	4.11	2.09
2011	0.49	0.36	0.46	4.50	2.61
2012	0.62	0.32	0.51	6.71	2.31
2013	0.71	0.29	0.54	6.98	3.68
2014	0.18	0.26	0.57	7.73	0.72
2015	0.28	0.34	0.60	7.14	1.41
2016	1.36	0.3	0.85	7.68	3.9
Total	4.52	4.89	5.62	71.31	22.05

DDD – defined daily dose; ATC – anatomical-therapeutic-chemical classification.

These variations in consumption can be attributed to the trend of prescribing new fixed combinations, such as travoprost/timolol, dorzolamide/timolol, timolol/brimonidine, etc, and an individual approach to the glaucoma treatment. The highest consumption of drugs in treatment of glaucoma according to DDD/1,000 inhabitants/day in all ATC groups, except for the S01EB group was registered in 2016. An individual analysis showed that the consumption of adrenomimetics and prostaglandin analogues was increasing until 2014, after which the significant fall was registered (both drugs represent an initial/effective therapy, either alone, or in the combination). At the same time, the consumption of carbonic anhydrase inhibitors had a linear rise (due to the prescriptions related to all types of glaucoma, including the secondary glaucoma), while parasympathomimetics were in linear decrease (due to the prescriptions of the medicaments mainly for the angular glaucoma).

In Slovenia, the linear increase in total consumption of forcible drugs in the period 2006–2017 was observed. An analysis of DDD/1,000 inhabitants/day indicated variations in relation to the ATC group. The highest increase in consumption was recorded in the S01EE group, while the consumption of sympathomimetics increased until 2016, after which there was a slight decline. The fall in consumption was seen in the S01EB group, while the consumption of S01EC and S01ED groups after 2011 was relatively stable<sup>13</sup>. In Norway, in the period 2011–2015, there was a linear decrease in the total consumption of drugs from the S01E-ATC group, observed through DDD/1,000 inhabitants/day. A mo-

re detailed analysis by groups showed that consumption of S01EA, S01EC and S01EE was growing, while consumption of S01ED decreased. At the same time, after 2013, the consumption of parasympathomimetics was relatively constant and stable<sup>14</sup>.

The first line of treatment for glaucoma, regardless of the general rule that the therapy should be individualized taking into account the patient's comorbidity, its accompanying therapy, the existence of the contraindication for the use of some anti-glaucoma drugs, belongs to prostaglandin analogues. If glaucoma patients have an additional eye inflammation, then they should not take prostaglandin analogues, and advantage should be given to beta-blockers, or to another anti-glaucoma drugs. Our analysis indicated that the consumption of anti-glaucoma medicaments in Serbia is mainly in line with the modern recommendations on the treatment of glaucoma<sup>15</sup>.

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### Ilustracije

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

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

### Skraćenice i akronimi

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

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

**Detaljno uputstvo može se dobiti u redakciji ili na sajtu:**  
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