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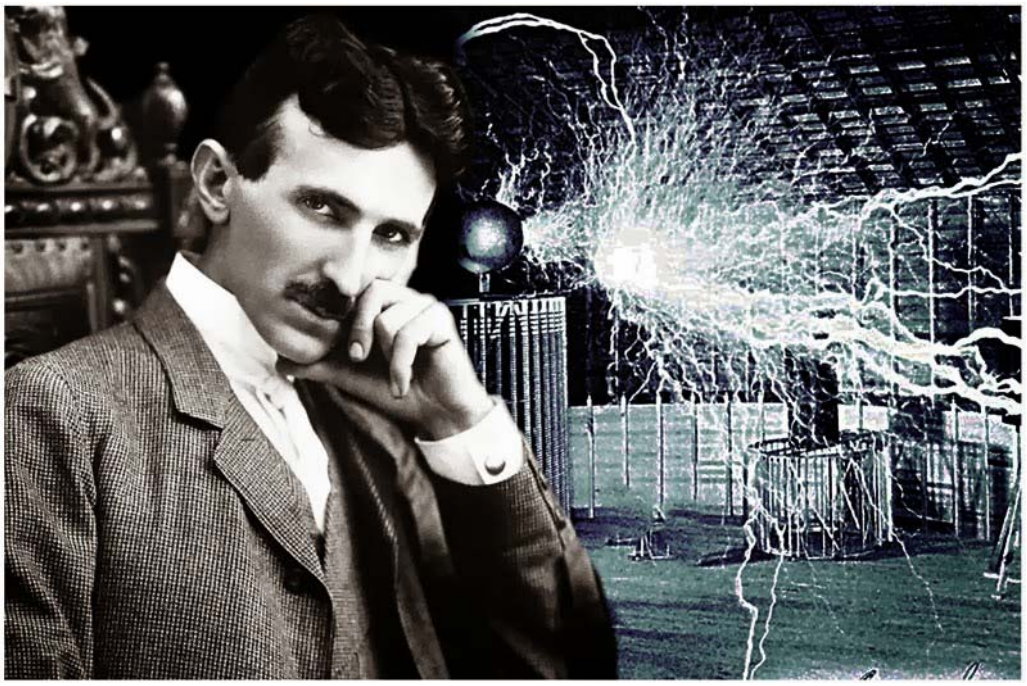

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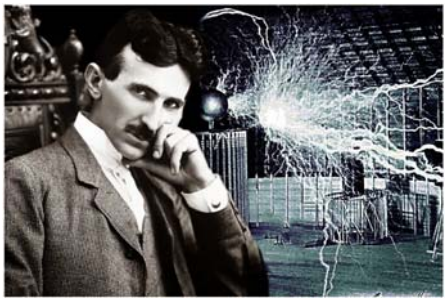

Nikola Tesla (July 10, 1856 – January 7, 1943), famous inventor and scientist of Serbian origin. This year, on July 10, marks the 160th anniversary of his birth.
Tesla is known primarily for his numerous inventions in the field of electrical engineering, although in other scientific fields left a deep mark, too. The Editorial of this issue of the *Vojnosanitetski Pregled* is an overview of his inventions important for medicine (see Editorial, p. 615–7).

Nikola Tesla (10. jul 1856 – 7. januar 1943), slavni pronalazač i naučnik srpskog porekla. Ove godine, 10. jula, navršava se 160 godina od njegovog rođenja.
Tesla je čuven, pre svega, po brojnim pronalascima u oblasti elektrotehnike, premda je i u drugim naučnim oblastima ostavio dubok trag. Uvodnik ovog broja Vojnosanitetskog pregleda predstavlja prikaz njegovih pronalazaka od značaja za medicinu (vidi Uvodnik, str. 615–7).

E D I T O R I A L / U V O D N I K

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Tesla's inventions of importance for medicine: reality or new visions of science (In honor of the 160th anniversary of the birth of Nikola Tesla)

Teslini izumi od značaja za medicinu: stvarnost ili novo gledanje na nauku (U čast 160-godišnjice rođenja Nikole Tesle)

Elizabeta Ristanović

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"*From time to time in the rare intervals, the great spirit of discovery finds itself on earth to communicate the mystery that will advance mankind. It selects the most able, the most deserving and whispers its secret into his ear. As a flash of light emerges valuable knowledge. While catching the hidden meaning the lucky one observes a magical change ... Miracles that he sees, although distant in time, will come to materialise. He knows it, there is no shred of doubt in his mind, in every fibre of his body he feels – it is a Great idea.*"

Nikola Tesla

This is not just another description about Nikola Tesla, the genius scientist of Serbian origin, who, as it was written in the history books, invented electricity, but this paper is also aimed at highlighting the lesser known parts of his creative activities whose results have been applied in medicine.

Nikola Tesla was born before 160 years, on July 10, 1856, in Smiljan near Gospic, Croatia and died on January 7, 1943, in New York, USA. He invented and patented the principle of the rotating magnetic field, a comprehensive system of production, transmission and utilization of multiphase alternating current (AC), the telephone amplifier, the induction motor, the generator of high frequency current (Tesla's electric current), Tesla's coil, wireless communications, radio, fluorescent light (aka. Tesla's electric arc) and about a thousand other inventions without which today's world would not be the same [1].

Visionary Tesla envisioned the TV, remote control cruise missiles, suspended trains with magnetic levitation, space weapons with high-energy rays, and even the Internet about which he dreamed as a "global single system network communications."

In the service of health: Tesla and medicine

Everything that he bestowed upon the world in the use of electricity is almost commonplace. However, Tesla's contribution to medicine is little known about.

The historians of Medical Sciences consider Tesla's most important contribution to this field the application of high-frequency and high voltage electrical currents. In an attempt to remove the sound vibrations that hindered the use of alternating current for the arc lamp, he constructed a high-frequency altenator, which, in turn, led him to the study of the properties of ultrasound. In the winter of 1891 Tesla revealed that rapid oscillation currents can cross the human body without causing any muscle spasms or tissue damage. Tesla claimed to have come to this conclusion by experimenting on his own body [2].

The scientific world was soon informed of the fascinating properties of Tesla's currents. In New York, London and Paris Tesla performed a famous experiment with a lighted pipe in his hand, having been exposed himself to currents of 50,000 volts. The experiment was echoed as a sensation and hinted at the possibility of therapeutic application of high frequency current and voltage. High-frequency currents are obtained using the so-called Tesla's coils, and his experimental evidence that they may pass through the human body without any danger opened a wide range of their applications in electrotherapy, magnetotherapy and thermotherapy as well as the possibilities for exploitation until now unexplored and insufficiently explained Tesla's radiant energy of high voltage electrostatic fields.

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Tesla was primarily interested in these currents (20–40 kHz) so as to solve the problem of lighting and remote transmission of electricity, but he noted that, using the aforementioned, without any danger can be warmed up tissues inside the human body [3].

At the lecture on the occasion of the annual meeting of the American Association for electrotherapy, in September 1898, Tesla synthesized all his papers relating to electrotherapy. He described the three groups of the physiological effects of high frequency currents. By conducting the experiment on his own body he determined that these currents could cause, in addition to the thermal effect, extreme tiredness, drowsiness and changes in breathing and blood circulation. In addition, he believed in the bactericidal power of his currents which all can be explained today by neutralizing free radicals.

Tesla, for example, had never been sick and he experienced a ripe old age, just because he was occasionally exposed to continual frequencies of low intensity that killed viruses and bacteria around him and in his body. It is thought that by using the devices that generate pulses of square shapes in certain frequencies Nikola Tesla managed to heal the famous chanteuse Edith Piaf by his currents. Doctor Rife from the United States, the first man on the planet who saw an alive virus under a microscope and then hit it by current of a certain frequency and a certain strength, wrotwe it all down on paper. A certain frequency that is resonant for the particular virus successfully killed it effectively and easily. The University of Southern California appointed a Special Medical Research Committee in 1934 to oversee the treatment being carried out by Dr. Rife. Earlier it took him many years and many hours of continuous work until he defined exactly the oscillations that were specific to each disease. A pioneer of this study was Nikola Tesla and his radio frequency (RF) electric apparatus Tesla's star [4, 5].

The second area of Tesla's research of interest for medicine were X-rays. He did experiments with tubes full of rarefied gas and electrode made of rubies, whereby he got an electron beam – X-rays known today. Contemporaries claimed that he was simply fascinated by these mysterious rays, but he had to abandon his experiments because of fire in his laboratory.

One of the first images using X-rays made in the United States, as stated in the "Phlogiston", a Journal of the History of Science, is the work of another physicist of Serbian origin, Mihajlo Pupin, a professor at Columbia University. According to this scientist's ingenious idea the "assault on the X-ray experiment resembles the onslaught of the West during the gold rush". A great deal of excitement was then present in the laboratories of Edison, Pupin, Herring, Frost, Thomson and Tesla. Only Tesla, however, used the great advantage of being able to adapt its high-frequency devices into powerful sources of X-rays. However, in an article published in 1896 on X-ray, he shows great respect for his wonderful discovery and describes the improved methods for obtaining X-rays [6].

Tesla later repeatedly touched upon the problems of general biology. The movement is for him the basics of life. The behaviour of beings, he argued, is fully determined by external factors. As a real engineer, Tesla analyzes the automatism of living beings. Without scientific education in physiology and biology he loses himself, especially at the end of his life, in metaphysical speculations that amazed the then public by their mixture of grandeur and naivety [7].

Nikola Tesla was fascinated by the properties of the human eye. Due to incorrect interpretation of a scientific text he believed in the existence of some kind of "fluorescence" within eye during brain activity. Tesla devoted much effort to his desire to photograph human thought, because he was convinced that intellectual activity faithfully reflects itself on the retina. He knew that the eye projected observed objects, but he was of the opinion that within the eye the thought is being transferred into an image. "The function of the eye", Tesla wrote in 1919, "is to project the image on the retina, and to communicate information to the optic nerve fibers. I believe that every thought, by means of a return action, causes a similar disorder, and that by the interpretation of this effect on the retina, the image can be projected onto the screen. This image is linked to the mind in exactly the same way as the original impression. We have available certain facts, but it is extremely difficult to perfect an instrument to achieve this task. As things stand, although I spent many years studying this problem, my main support in the work is still hope." This Tesla's letter was published in the book My findings. Later research based on the idea that Tesla was the first to publish, can be used to control the movement of people and to control their mind [8].

When it comes to electromagnetic effects on the human brain it is also known that the American physicist Bernard Istlund, using the ideas of Nikola Tesla, designed the system High-Frequency Active Auroral Research Project (HAARP) in Alaska, which under the name of the Institute for Research of Ionosphere works on extra low frequencies that are identical to the frequencies of brain waves, from 9.5 to 50 Hertz.

About the famous experiment by Nikola Tesla, where he participated and which also witnessed the Unated States (US) military, was used the resonant alignment with the impulses of the brain and the so-called. neurolinguistic programming, which is, in fact, a prepared "conversation" with the subconscious of gathered persons. Such examples demonstrate the power of thought and the existence of the connection between the subconscious and consciousness in human reasoning [9].

Instead of the conclusion: new visions and new truths

Nikola Tesla certainly is one of the world's most brilliant inventors. Tesla was not only the inventor of new type apparatuses, the inventor, but the creator of new truths and new principles. Tesla said that the most important product of the creating mind is an invention, and its ultimate goal was to master nature by one's mind and exploitation of its forces for the needs of humanity. "It was recognised that I was one of the largest workers insofar as the thought is equivalent to work, because I devoted to it almost all my waking hours" , said Tesla. If someone removed his work, the whole world would remain in the dark, and the largest number of machines, mobile and fixed, would stop functioning. Hence the name of this ingenious planetary scientist shines today in its brightest splendour.

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ORIGINAL ARTICLES

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Optical coherence tomography in the evaluation of structural changes in primary open-angle glaucoma with and without elevated intraocular pressure

Optička koherentna tomografija u proceni strukturnih promena kod primarnog glaukoma otvorenog ugla sa i bez povišenog intraokularnog pritiska

Marija Trenkić Božinović*, Gordana Zlatanović*†, Predrag Jovanović*†, Dragan Veselinović*†, Jasmina Djordjević Jocić*†, Marija Radenković*, Mirko Resan‡§

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Abstract

Background/Aim. Glaucoma is a progressive optic neuropathy characterized by damage of the retinal ganglion cells and their axons and glial cells. The aim of this study was to evaluate the differences and connections between changes in the visual field and the thickness of the peripapillary retinal nerve fiber layer (RNFL), using optical coherence tomography (OCT) in patients with primary open-angle glaucoma with normal and elevated intraocular pressure (IOP). **Methods.** This prospective study included 38 patients (38 eyes) with primary open-angle glaucoma with normal intraocular pressure (NTG) and 50 patients (50 eyes) with primary open-angle glaucoma with elevated intraocular pressure (HTG), paired by the same degree of structural glaucomatous changes in the optic nerve head and by age. OCT protocols 'fast RNFL thickness' and 'fast optic disc' were used for testing. The patients' age, gender, best corrected visual acuity (BCVA), IOP, stereometric and functional parameters were compared. **Results.** The average age of the examined population was 65.49 ± 9.36 (range 44–83) years. There was no statistically significant difference by age and by gender between the two study groups ($p = 0.795$ and $p = 0.807$, respectively). BCVA was higher in patients with NTG but there was no statistically significant difference compared to HTG patients ($p = 0.160$). IOP was statistically significantly higher in patients with HTG compared to NTG patients (17.40 ± 2.77 mmHg *vs* 14.95 ± 3.01 mmHg, $p = 0.009$). The cup/disc (C/D) ($p = 0.258$), mean deviation (MD) ($p = 0.477$), corrected patern standard deviation (CPSD) ($p = 0.943$), disk area ($p = 0.515$), rim area ($p = 0.294$), rim volume ($p = 0.118$), C/D area R ($p = 0.103$), RNFL Average ($p = 0.632$), RNFL Superior ($p = 0.283$) and RNFL Inferior ($p = 0.488$) were not statistically significantly different between the groups. **Conclusion.** OCT measurements of the RNFL thickness provide clinically significant information in monitoring of glaucomatous changes. There are no differences in the patterns of RNFL defects *per* sectors and quadrants between NTG and HTG, measured by OCT.

Key words:
glaucoma, open-angle; intraocular pressure; tomography, optical coherence.

Apstrakt

Uvod/Cilj. Glaukom je progresivna optička neuropatija koja oštećuje retinalne ganglijske ćelije i njihove aksone i glijalne ćelije. Cilj rada bio je da se procene razlike i veze između promena u vidnom polju i debljine peripapilarnih nervnih vlakana retine (RNFL), uz pomoć optičke koherentne tomografije (OCT), kod obolelih od primarnog glaukoma otvorenog ugla sa normalnim i povišenim intraokularnim pritiskom (IOP). **Metode.** U ovu prospektivnu studiju bilo je uključeno 38 bolesnika (38 očiju) obolelih od primarnog glaukoma otvorenog ugla sa normalnim intraokularnim pritiskom (NTG) i 50 bolesnika (50 očiju) obolelih od primarnog glaukoma otvorenog ugla sa povišenim intraokularnim pritiskom (HTG). Bolesnici su bili upareni po stepenu glaukomnih strukturnih promena glave vidnog živca i godinama starosti. Za ispitivanje su korišćeni '*fast RNFL thickness*' i '*fast optic disc*' OCT protokoli. Upoređivani su starost bolesnika, pol, najbolje korigovana vidna oštrina (BCVA), IOP, stereometrijski i funkcionalni parametri. **Rezultati.** Prosečna starost ispitanika bila je

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65,49 ± 9,36 (opseg 44–83) godine. Utvrđeno je da ne postoji statistički značajna razlika prema starosti i prema polu između dve ispitivane grupe (p = 0,795, odnosno p = 0,807). BCVA je bila veća kod bolesnika sa NTG, ali nije bilo statistički značajne razlike u odnosu na HTG bolesnike (p = 0,160). IOP je bio statistički značajno viši kod bolesnika sa HTG u odnosu na NTG bolesnike (17,40 ± 2,77 mmHg *vs* 14,95 ± 3,01 mmHg, p = 0,009). Vrednosti *cup/disc* (C/D) (p = 0,258), *mean deviation* (MD) (p = 0,477), *corrected pattern standard deviation* (CPSD) (p = 0,943), *disk area* (p = 0,515), *rim area* (p = 0,294), *rim volume* (p = 0,118), C/D *area* R (p = 0,103), RNFL *Average* (p = 0,632), RNFL *Superior* (p = 0,283) i RNFL *Inferior* (p = 0,488) nisu se statistički značajno razlikovale između ispitivanih grupa. **Zaključak.** Pomoću OCT dobijeni parametri debljine RNFL obezbeđuju klinički važne informacije u praćenju glaukomnih promena. Ne postoje razlike u defektu RNFL po sektorima i kvadrantima između NTG i HTG bolesnika merenih OCT procedurom.

Ključne reči:
glaukom, otvorenog ugla; intraokularni pritisak; tomografija, optička, koherentna.

Introduction

Glaucoma is a progressive optic neuropathy characterized by damage of the retinal ganglion cells and their axons and glial cells [1–3]. This leads to morphological changes of the optic nerve head (ONH) and the accompanying changes in the visual field [4]. Primary open-angle glaucoma (POAG) is the most common glaucoma type. Although, elevated intraocular pressure (IOP) is considered as one of the main causes of the occurrence of glaucoma, POAG in elderly patiens may occur with elevated intraocular pressure (HTG), normal intraocular pressure (NTG) as well as ocular hypertension (OH) [5, 6]. Despite the numerous technical possibilities for testing the morphological and functional changes in glaucoma, POAG with and POAG without elevated IOP is a field in which many scientific results and opinions are opposite. Numerous studies, both past and present, have shown that the structural and functional changes in patients with HTG and NTG are almost identical [7–11], as well as being completely different [12–22].

Optical coherence tomography (OCT) is a contactless, non-invasive diagnostic procedure, that can be repeated number of times [23–27]. OCT provides measurements of the thickness of the retinal nerve fiber layer, the loss of 25% of which, occurs in the early stages of glaucoma [4, 28]. Some authors have shown that the loss of as much as 40% to 50% of the nerve fibers of the optic nerve does not have to be accompanied by changes in the visual field [28]. Because of this change, RNFL thickness is considered as the most sensitive indicator for glaucomatous damage [29].

Yamazaki et al. [30], and Woo et al. [31] demonstrated the different patterns of retinal nerve fiber layer (RNFL) defects in patients with HTG compared to patients with NTG, ie. in NTG the defects are more localized. However, Kubota et al. [32], and Kook et al. [33] reported that there was a symmetrical loss of RNFL in the superior and inferior quadrants in POAG patients with elevated IOP. In contrast to all this, Mok et al. [1, 34] reported that there are no differences in RNFL thinning pattern in HTG and NTG; it was a mixture of localized and diffuse RNFL defects in both cases [29].

The aim of this study was to evaluate the differences and connections between changes in the visual field and the thickness of the peripapillary retinal nerve fiber layer, measured by OCT in POAG patients with normal and elevated IOP.

Methods

This prospective study included 38 eyes (38 patients) suffering from POAG with normal IOP (NTG) and 50 eyes (50 patients) suffering from POAG with elevated IOP (HTG), paired by the degree of structural glaucomatous changes of the optic nerve head and by age. The selection and monitoring of the patients was performed at the Department of Ophthalmology, University Medical Center Niš. The research followed the Declaration of Helsinki and was approved by the Ethical Review Board of Clinical Center Niš.

All of the patients had the previous diagnosis of POAG with elevated or normal IOP. Exclusion criteria were: spherical equivalent > ± 4 Dsph, other ocular diseases, ocular surgery performed in the previous year, laser treatment, trauma, secondary glaucoma, neurological diseases with changes in the visual field, patients with unreliable visual field (defined as false-negative errors >33%, false-positive errors > 33%, and fixation losses > 20%), patients with anomalies of the optic nerve head. If both eyes of the patients satisfied the inclusion criteria, only one eye was randomly selected.

The following demographic and stereometric parameters were measured and studied here: the patients' age (years); gender; best corrected visual acuity (BCVA) determined by Snellen; IOP (in mmHg) measured by applanation tonometry; the size of the optic nerve head excavation (cup/disc - C/D) determined by indirect ophthalmoscopy with 90 D lens; the value of mean deviation (MD, in dB) and corrected pattern standard deviation (CPSD, in dB) obtained by computed (static) perimetry; disc area (in mm^2), rim area (in mm^2), rim volume (in mm^3), C/D area ratio, the global average thickness of peripapillary retinal nerve fibers (RNFL Avg, in μm) and average RNFL thickness (in μm) in four quadrants and all sectors measured by OCT.

Static perimetry, Humphrey visual field analyzer (Carl Zeiss Meditec, Inc., treshold test 24-2), was used to reveal the defects in the visual field of patients. The first visual field was never considered with regard to the learning curve.

OCT measurements were performed on the Stratus OCT (Carl Zeiss Meditec, Inc.). For, testing, 'fast RNFL thickness' and 'fast optic disc' OCT protocols were used. Fast RNFL protocol consists of three circular scans, each 3.46 mm in diameter, centered on the optic disc. This diameter has been shown to be optimal for RNFL thickness analysis

and provides better reproducibility than single scan. Scans were further analyzed using the RNFL thickness average analysis protocol (OU). The protocol quantifies the average RNFL thickness (360° measure), as well as 12 clock-hour sectoral measures (30°): 1 clock-hour: supero-nasal (15 to 45°), 2: nasal-superior (45 to 75°), 3: nasal (75–105°), 4: nasal-inferior (105–135°), 5: inferonasal (135–165°), 6: inferior (165–195°), 7: inferotemporal (195–225°), 8: tempo-inferior (225–255°), 9: temporal (255–285°), 10: tempo-superior (285–315°), 11: superotemporal (315–345°), 12: superior (345–15°). Three adjacent measures are then averaged (clock hours 11, 12, 1; 2, 3, 4; 5, 6, 7; 8, 9, 10) to provide four quadrant thickness values. One author performed all the images and measurements.

The data are shown as arithmetic means and standard deviations. The comparison of the values between patients with NTG and HTG was carried out using *t*-test or Mann-Whiteney test. The association of these parameters was tested by Spearman's correlation coefficient. Statistical analysis of data was performed by SPSS (version 16.0, SPSS Science, Chicago, IL, USA). *p*-value < 0.05 was considered statistically significant.

Results

The study included 38 (43.18%) NTG patients and 50 (56.82%) HTG patients. The average age of the examined population was 65.49 ± 9.36 (range 44–83) years. No statistically significant difference by age and by gender was found between the two study groups ($p = 0.795$ and $p = 0.807$, respectively) (Table 1).

In the group of NTG patients, the therapy of 32 (84.21%) patients included one anti-glaucomatous topical medication (81.25% α-agonist, 18.75% carbonic anhydrase inhibitor), four patients (10.53%) received two medications, and two patients (5.26%) received three medications. In the second group, HTG patients were on the following therapy: 12 (24.00%) patients received one medication, 16 (32.%) patients two medications, and 22 (44%) patients three medications.

Best corrected visual acuity was higher in patients with NTG but there was no statistically significant difference compared to HTG patients ($p = 0.160$). IOP with the prescribed therapy was significantly higher in patients with HTG compared to NTG patients (17.40 ± 2.77 mmHg *vs* 14.95 ± 3.01 mmHg, $p = 0.009$). The morphological parameters of the optic nerve head, due to the examination of the fundus with the 90D lens, C/D, were not statistically significantly different between the patients with HTG and NTG ($p = 0.258$). Changes in the visual field, ie. functional parameters: MD ($p = 0.477$) and CPSD ($p = 0.943$) did not show any statistical significance. Stereometric ONH parameters obtained by OCT: disk area ($p = 0.515$), rim area ($p = 0.294$), rim volume ($p = 0.118$), C/D area R ($p = 0.103$), RNFL Avg ($p = 0.632$), RNFL Superior (RNFL Sup) ($p = 0.283$) and RNFL Inferior (RNFL Inf) ($p = 0.488$) were not statistically significantly different between the groups (Table 1, Figure 1).

Table 1

Demographic and stereometric parameters of primary open-angle glaucoma (POAG) of the patients with normal intraocular pressure (NTG) and elevated intraocular pressure (HTG)

Parameters	NTG (n = 38) $\bar{x} \pm SD$	HTG (n = 50) $\bar{x} \pm SD$	t/z*	*p*
Age (years)	65.90 ± 9.22	65.16 ± 9.65	0.262	0.795
Gender (M/F), n	15/23	16/34	0.059	0.807
BCVA	0.89 ± 0.23	0.81 ± 0.26	1.406*	0.160
IOP (mmHg)	14.95 ± 3.01	17.40 ± 2.77	2.772	0.009
C/D	0.51 ± 0.13	0.46 ± 0.16	1.146	0.258
MD (dB)	-5.35 ± 3.31	-6.16 ± 2.90	0.711*	0.477
CPSD (dB)	4.10 ± 3.45	3.86 ± 2.90	0.071*	0.943
Disk area (mm^2)	2.53 ± 0.44	2.44 ± 0.46	0.658	0.515
Rim area (mm^2)	1.36 ± 0.37	1.48 ± 0.38	1.063	0.294
Rim volume (mm^3)	0.26 ± 0.11	0.34 ± 0.24	1.603	0.118
C/D area R	0.46 ± 0.14	0.37 ± 0.19	1.665	0.103
RNFL Avg (μm)	92.74 ± 17.91	95.37 ± 17.87	0.483	0.632
RNFL Sup (μm)	105.11 ± 30.27	114.72 ± 27.22	1.090	0.283
RNFL Inf (μm)	123.84 ± 28.30	117.84 ± 27.95	0.701	0.488
RNFL Nas (μm)	80.05 ± 21.58	82.16 ± 22.06	0.457	0.648
RNFL Temp (μm)	64.20 ± 15.30	62.12 ± 10.74	0.709	0.478

M/F – male/female; BCVA – best corrected visual acuity; IOP – intraocular pressure; MD – mean deviation; CPSD – corrected pattern standard deviation; C/D area R – cup/disc area ratio; RNFL – retinal nerve fiber layer; RNFL Avg – RNFL average; RNFL Sup – RNFL superior; RNFL Inf – RNFL inferior; RNFL Nas – RNFL nasal; RNFL Temp – RNFL temporal.

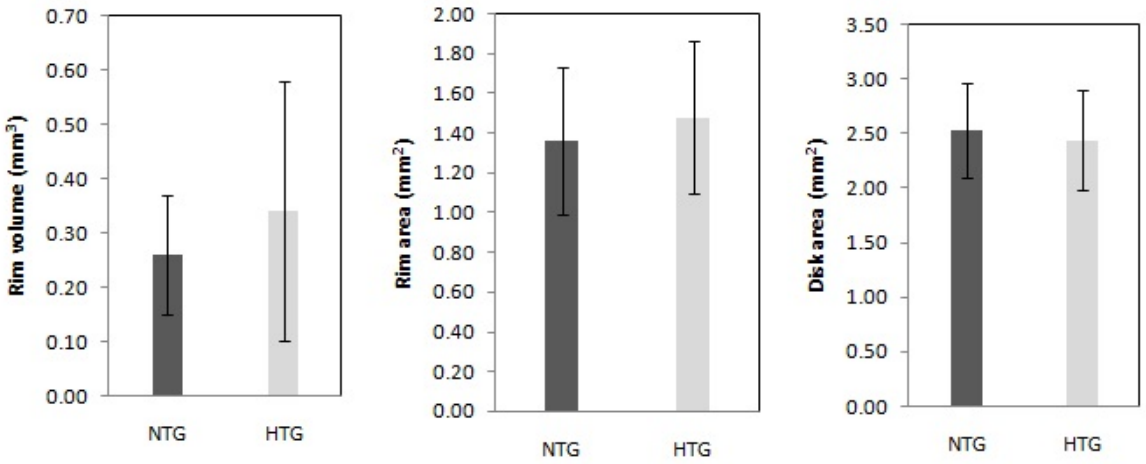

Fig. 1 – Rim volume, rim area and disc area in normal intraocular pressure (NTG) and elevated intraocular pressure (HTG) patients showing no statistically significant difference between NTG and HTG groups. (rim volume: $p = 0.118$; rim area: $p = 0.294$; disc area: $p = 0.515$).

The average global and quadrant, and sectoral RNFL thickness values were compared between the groups of NTG and HTG patients; no statistically significant difference in the values of the thickness of RNFL as *per* quadrant and sectors was found (Tables 1 and 2).

The correlation analysis of the structural and functional parameters in patients with NTG and HTG was performed by Spearman's correlation coefficient (Tables 3 and 4). A statistically significant positive correlation in the group of NTG patients was found between RNFL Avg and rim volume ($p = 0.713$, $p = 0.001$), RNFL Avg and rim area ($p = 0.847$, $p < 0.001$), RNFL Sup and rim area ($p = 0.522$, $p = 0.022$), RNFL Inf and RNFL Nasal (RNFL Nas) ($p = 0.595$, $p < 0.001$), RNFL Inf and RNFL Temporal (RFNL Temp) ($p = 0.574$, $p = 0.010$), RNFL Inf and rim volume ($p = 0.462$, $p = 0.047$), RNFL Inf and rim area ($p = 0.674$, $p = 0.002$), RNFL Nas and rim volume ($p = 0.702$, $p = 0.001$), RNFL Nas and rim area ($p = 0.547$, $p = 0.015$), RNFL Temp and rim area ($p = 0.651$, $p = 0.003$), RNFL Avg and disk area ($p = 0.634$, $p = 0.004$), RNFL Sup and disk area ($p = 0.778$, $p < 0.005$) (Table 3).

A statistically significant positive correlation in the group of HTG patients was found between RNFL Avg and rim volume ($p = 0.627$, $p = 0.001$), RNFL Avg and rim area ($p = 0.467$, $p = 0.019$), RNFL Sup and RNFL Nas ($p = 0.420$, $p = 0.036$), RNFL Sup and rim volume ($p = 0.484$, $p = 0.014$), RNFL Inf and RNFL Nas ($p = 0.594$, $p = 0.002$),

Table 2

The thickness (μm) of the peripapillary retinal nerve fiber layer (RNFL) in the normal intraocular pressure (NTG) and elevated intraocular pressure (HTG) patients on optical coherence tomography (OCT)

Clock-hour sectoral measures	NTG (n = 38) $\bar{x} \pm SD$	HTG (n = 50) $\bar{x} \pm SD$	p*
1	93.70 ± 30.58	109.64 ± 32.49	0.100
2	88.75 ± 23.72	91.92 ± 26.02	0.631
3	64.40 ± 21.12	69.04 ± 18.89	0.411
4	82.05 ± 26.09	85.40 ± 28.78	0.568
5	110.80 ± 28.18	107.52 ± 35.37	0.706
6	132.65 ± 40.58	121.76 ± 34.86	0.723
7	123.55 ± 46.18	115.48 ± 24.94	0.213
8	65.65 ± 21.32	62.64 ± 11.42	0.267
9	49.00 ± 14.26	49.60 ± 9.80	0.936
10	74.85 ± 20.64	73.72 ± 15.48	0.973
11	111.20 ± 34.75	115.32 ± 24.51	0.749
12	107.55 ± 39.89	121.32 ± 37.24	0.320

***Mann-Whitney U test.**

Clock-hour sectoral measures: 1 – supero-nasal (15 to 45°); 2 – nasal-superior (45 to 75°); 3 – nasal (75–105°); 4 – nasal-inferior (105–135°); 5 – inferonasal (135–165°); 6 – inferior (165–195°); 7 – inferotemporal (195–225°); 8 – tempo-inferior (225–255°); 9 – temporal (255–285°); 10 – tempo-superior (285–315°); 11 – superotemporal (315–345°); 12 – superior (345–15°).

Table 3

Correlation analysis of the structural and functional parameters in the patients with normal intraocular pressure (NTG)

Parameters	Rim volume	Rim area	Disk area	MD	CPSD
RNFL Avg	**0.713****	**0.847****	**0.634****	0.450	-0.166‡
p	**0.001**	**< 0.001**	**0.004**	0.053	0.496†
RNFL Sup	0.269	**0.522***	**0.778****	0.400	-0.218
p	0.266	**0.022**	**< 0.001**	0.090	0.369
RNFL Inf	**0.462***	**0.674****	0.158	0.450	-0.111
p	**0.047**	**0.002**	0.519	0.053	0.651
RNFL Nas	**0.702****	**0.547****	0.161	0.099	0.187
p	**0.001**	**0.015**	0.499	0.686	0.443
RNFL Temp	0.437	**0.651****	-0.059	0.340	-0.246
p	0.061	**0.003**	0.810	0.155	0.310
RIM VOLUME	-	**0.729****	**0.551***	0.059	0.060
p		**< 0.001**	**0.015**	0.811	0.808
RIM AREA		-	0.293	0.449	-0.307
p			0.223	0.054	0.201
MD			0.279	-	**-0.564***
p			0.247		**0.012**

Data are presented as Spearman's coefficient; * – statistically significant; ** – statistically highly significant.

RNFL – retinal nerve fiber layer; RNFL Avg – RNFL-Average; RNFL Sup – RNFL-Superior; RNFL Inf – RNFL Inferior; RNFL Nas – RNFL-Nasal; RNFL Temp – RNFL – Temporal; MD – mean deviation; CPSD – corrected pattern standard deviation.

Table 4

Correlation analysis of the studied parameters in the patients with elevated intraocular pressure (HTG)

Parameters	Rim volume	Rim area	Disk area	MD	CPSD
RNFL Avg	**0.627****	**0.467***	**-0.543****	0.172	0.086
p	**0.001**	**0.019**	**0.005**	0.410	0.682
RNFL Sup	**0.484***	0.285	**-0.610****	-0.126	0.279
p	**0.014**	0.167	**0.001**	0.550	0.176
RNFL Inf	**0.508****	0.377	**-0.462***	0.348	-0.158
p	**0.010**	0.064	**0.019**	0.088	0.451
RNFL Nas	**0.422***	**0.441***	-0.192	0.140	0.135
p	**0.035**	**0.027**	0.358	0.505	0.521
RNFL Temp	0.257	0.346	-0.121	**0.454***	**-0.470***
p	0.214	0.090	0.566	**0.022**	**0.018**
Rim volume	-	**0.746****	-0.018	-0.072	0.149
p		**< 0.001**	0.930	0.734	0.477
Rim area		-	-0.379	0.190	0.027
p			0.061	0.363	0.897
MD			-0.136	-	**-0.475***
p			0.516		**0.016**

For explanation see under Table 3.

RNFL Inf and RNFL Temp ($\rho = 0.418$, $p = 0.038$), RNFL Inf and rim volume ($\rho = 0.508$, $p = 0.010$), RNFL Nas and rim volume ($\rho = 0.422$, $p = 0.035$), RNFL Nas and rim area ($\rho = 0.441$, $p = 0.027$), RNFL Temp and MD ($\rho = 0.454$, $p = 0.022$). The strongest correlation was found between RNFL Avg and RNFL Inf ($\rho = 0.801$, $p < 0.001$). A statistically significant negative correlation was found between RNFL Temp and CPSD ($\rho = -0.470$, $p = 0.018$), RNFL Avg and disk area ($\rho = -0.543$, $p = 0.005$), RNFL Sup and disk area ($\rho = -0.610$, $p = 0.001$), RNFL Inf and disk area ($\rho = -0.462$, $p = 0.019$) (Table 4).

The strongest correlation was found between RNFL Avg and rim area ($\rho = 0.847$, $p < 0.001$) in the patients with NTG. This correlation was also statistically significant in pa-

tients with HTG, RNFL Avg and rim area (ρ = 0.467, p = 0.019) (Figure 2). The correlation between RNFL Avg thickness and rim volume showed statistical significance in both groups of patients (NTG: ρ = 0.713, p = 0.001, HTG: ρ = 0.627, p = 0.001) (Figure 3).

The study of correlation between changes in the visual field MD and CPSD with RNFL Avg thickness and rim area in glaucomatous eyes did not find statistical significance in any of the groups of patients with POAG (Figures 4 and 5). Only statistically significant correlation existed between RNFL Temp and MD (ρ = 0.454, p = 0.022) and between RNFL Temp and CPSD (ρ = -0.470, p = 0.018) in the HTG patients.

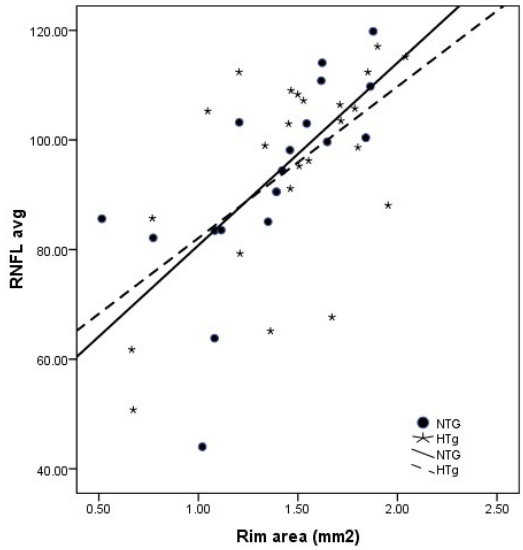

Fig. 2 – Thickness of the retinal nerve fiber layer average (RNFL Avg) (µm) plotted *versus* the rim area (mm^2) for the normal intraocular pressure (NTG) and elevated intraocular pressure (HTG) patients (NTG: ρ = 0.847, p < 0.001; HTG: ρ = 0.467, p = 0.019).

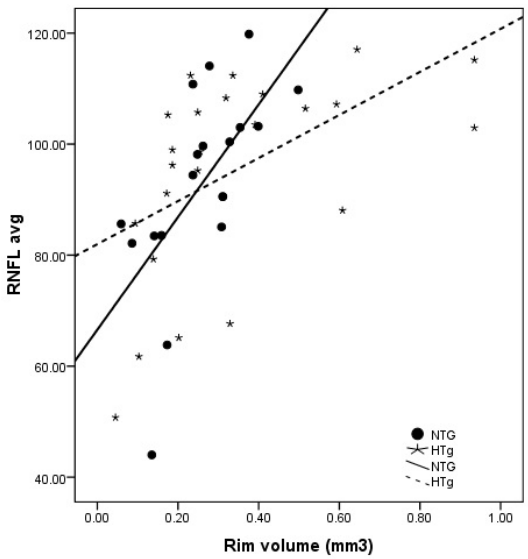

Fig. 3 – Thickness of the retinal nerve fiber layer average (RNFL Avg) (µm) plotted *versus* the rim volume (mm^3) for the normal intraocular pressure (NTG) and elevated intraocular pressure (HTG) patients (NTG: ρ = 0.713, p = 0.001; HTG: ρ = 0.627, p = 0.001).

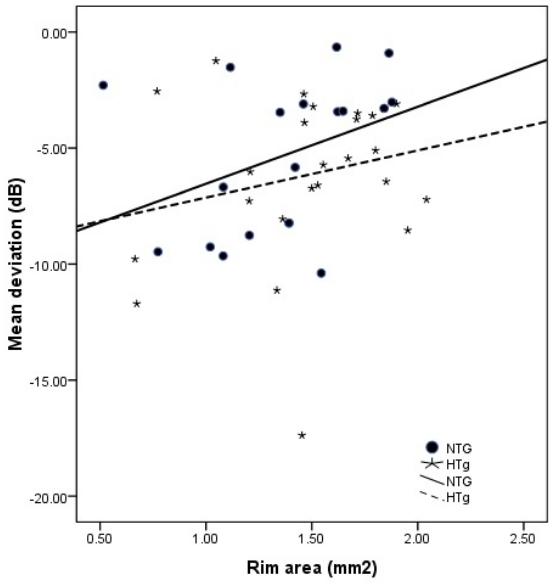

Fig. 4 – Mean deviation (MD) in dB plotted *versus* the rim area for the normal intraocular pressure (NTG) and elevated intraocular pressure (HTG) patients (NTG: ρ = 0.449, p = 0.054; HTG: ρ = 0.190, p = 0.363).

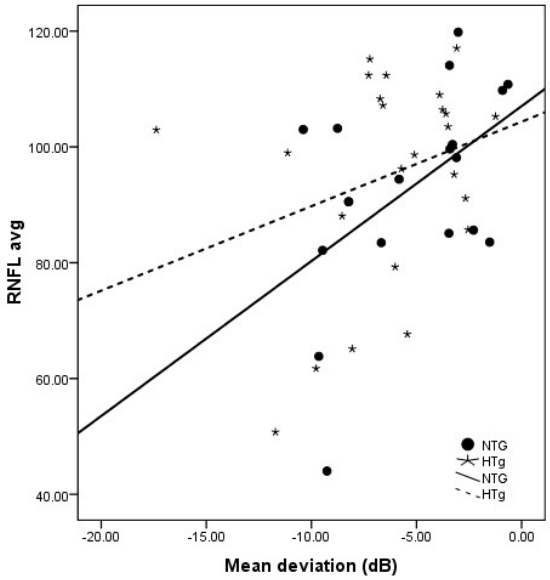

Fig. 5 – Thickness of the retinal nerve fiber layer average (RNFL Avg) (µm) plotted *versus* the mean deviation (MD, dB) for normal intraocular pressure (NTG) and elevated intraocular pressure (HTG) patients (NTG: ρ = 0.450, p = 0.053; HTG: ρ = 0.172, p = 0.410).

Discussion

Glaucoma is associated with the loss of retinal ganglion cells, which manifests as defects in the RNFL. The most important part of glaucoma diagnosis is the evaluation of the thickness of the retinal nerve fibers and changes of the optic disc. This can be assessed by OCT, as a good, objective and repeatable method. Also, OCT with its high axial resolution of 9–10 μm is a much superior method than GDx VCC and Heidelberg retina tomograph (HRT) III [1, 29]. For a long time it was thought that there are different patterns of RNFL thinning occurring in patients with POAG with elevated IOP and without elevated IOP. Kubota et al. [32] found a difference in RNFL defects in patients with HTG and NTG. RNFL thickness was reduced symmetrically in the superior and inferior quadrants in HTG patients, whereas in patients with NTG a more localized RNFL loss in inferior quadrant occurs [32, 35]. Quite different results have appeared in recent years: there are no differences in RNFL thinning pattern between HTG and NTG, measured by OCT [29]. Our research also shows no differences in the defect pattern (localized or diffuse) of RNFL in HTG and NTG patients, and no differences in the amount of lost retinal nerve fibers of the two groups of patients. The strength of this statement is supplemented by the fact that the patients were at the same disease stage, with the same degree of structural damage of the optic nerve head and the same degree of change in the visual field (MD < 12 dB). In addition, all glaucoma patients had spherical equivalent (SE) within ± 4 Dsph, so the possibility of involving patients with very large or small optic nerve head (ONH) was reduced to a minimum. Our results are consistent with Mok et al. [1, 34], and Konstantakopoulou et al. [29] published results, that patients with HTG and NTG showed a similar RNFL thinning pattern, which is a mixture of diffuse and localized changes, measured by OCT [36].

Studying the localized RNFL loss by clock-hour sectors did not show any statistically significant difference, although this method provides more precise information on smaller fields of peripapillary nerve fiber loss. Furthermore, OCT paremeters for inferotemporal and superotemporal sector, which are considered to be of great help for the early diagnosis of glaucoma and making a difference compared to healthy eyes, did not differ significantly between our groups of HTG and NTG patients [1, 37]. The study did not include patients with ocular hypertension as well as a healthy population because the differences between these two groups of patients with POAG patients are already tested and proven.

The results from this study related to age, gender, BCVA and morphological parameters of the optic nerve head did not differ significantly between the patients with HTG and NTG, and are fully in line with other publications [2, 29, 34, 38]. Only the IOP was significantly lower in patients with NTG ($p = 0.009$) [2]. Häntzschel et al. [2] reported that visual acuity was significantly better in patients with NTG than in patients with HTG ($p < 0.002$); we found that BCVA was higher in patients with NTG but there was no statistically significant difference compared to HTG patients ($p = 0.160$), most likely due to better randomization. The sample size for this study is relatively small (38 NTG, 50 HTG), but it is in accordance with the groups studied by other authors: Mok et al. [34] (38 NTG, 48 HTG), and Konstantakopoulou et al. [29] (20 NTG, 21 HTG).

Sihota et al. [28] found that defects in the visual field (MD) positively correlate with the thickness of RNFL superior, inferior, nasal, temporal and average, and that there is a negative correlation between RNFL (average, superior, nasal, inferior, temporal) parameters with CPSD. Shin et al. [38] reported the connection between MD and CPSD and RNFL Avg and RNFL Inf thickness parameters. Our results of two visual field indices (MD and CPSD) showed that there is a statistically significant positive correlation between RNFL Temp and MD ($\rho = 0.454$, $p = 0.022$) and negative correlation between RNFL Temp and CPSD ($\rho = -0.470$, $p = 0.018$), only in patients with HTG. This difference could be due to the difference in sample size and because the other authors compared the patients with POAG and healthy population [28].

Conclusion

OCT measurements of RNFL thickness provide clinically significant information in the monitoring of glaucomatous changes. There are no differences in RNFL thinning pattern *per* sectors and quadrants between NTG and HTG, as assessed by OCT. However, further larger studies are needed to supplement these findings view of the fact that it is known that RNFL thickness measurements and morphology of ONH have wide inter-individual and interracial variations.

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Neobladder "Belgrade pouch": Metabolic consideration

Neobešika *Belgrade pouch*: metabolička razmatranja

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Abstract

Background/Aim. The ileal neobladder should be a low-pressure reservoir with acceptable volume and relatively small resorptive surface. A larger inner surface of the ileal pouch is associated with the high resorption of urine metabolites through intestinal mucosa and systemic metabolic disturbances, while a too small pouch results in a higher frequency of voiding and incontinency. The aim of this study was to investigate it is possible to create a neobladder from a shorter ileal segment compared to standard surgical techniques, and reduce metabolic complications. **Methods.** This prospective study included 77 male patients, scheduled for radical cystectomy and orthotopic neobladder derivation. The patients were divided into two groups: the standard pouch (SP) group of 37 patients scheduled for standard orthotopic neobladder, using a 50−70 cm long terminal ileum segment; the "Belgrade pouch" (BP) group of 40 patients scheduled for original, orthotopic urinary reservoir, using a 25−35 cm long terminal ileum segment. We measured neobladder capacity, acidosis, base excess and bicarbonate concentration in the postoperative month 3rd, 6th, 12th and 15th. **Results.** At the end of the study, the patients from the SP group had much higher neobladder capacity than the natural bladder − 750 mL (range 514−2,050 mL); in contrast, the patients from the BP group had average capacity of 438 mL (range 205−653 mL) ($p < 0.001$). At the end of the study, there were more patients with acidosis (37.8% : 2.5%), base excess (35.1% : 7.5%) and low bicarbonate level (40.5% : 20.0%) in the SP group, than in the BP group, respectively ($p < 0.001$). **Conclusion.** "Belgrade pouch", make from 25−35 cm long terminal ileum segment may obtain adequate capacity and lower rate of metabolic disturbances than standard, high capacity orthotopic neobladders.

Key words:
urinary bladder neoplasms; urologic surgical procedures; colonic pouches; serbia; metabolism.

Apstrakt

Uvod/Cilj. Ilealna neobešika trebalo bi da bude rezervoar niskog pritiska, adekvatnog kapaciteta i relativno male resorptivne površine. Velika unutrašnja površina neobešike napravljene od tankog creva rezultuje apsorpcijom urina kroz crevnu sluznicu neobešike, što može da dovede do ozbiljnih sistemskih metaboličkih komplikacija. Sa druge strane, izrada neobešike malog kapaciteta može da rezultuje učestalim mokrenjem i inkontinencijom. Cilj ove studije bio je da se utvrdi da li je moguće napraviti neobešiku od kraćeg crevnog segmenta u odnosu na standardne tehnike i time smanjiti metaboličke postoperativne komplikacije. **Metode.** U ovu prospektivnu studiju bilo je uključeno 77 bolesnika muškog pola, koji su bili podvrgnuti radikalnoj cistektomiji i ortotopnoj ilealnoj derivaciji urina. Bolesnici su bili podeljeni u dve grupe: SP grupa koju je činilo 37 bolesnika standardnom ortotopnom neobešikom (*standard pouch*) od segmenta terminalnog ileuma dužine 50−70 cm; BP grupa od 40 bolesnika sa originalnom neobešikom ("*Belgrade pouch*") od kraćeg crevnog segmenta dužine 25−30 cm. Praćeni su sledeći parametri: kapacitet neobešike, acidoza, bazni eksces i koncentracija bikarbonatnih jona u krvi, 3, 6, 12 i 15 meseci nakon operacije. **Rezultati.** Na kraju studije, bolesnici iz SP grupe imali su značajno veći kapacitet neobešike [750 (514−2 050) mL)] od nativne mokraćne bešike i od neobešike BP grupe [438 (205−653 mL)] ($p < 0,001$). Takođe, na kraju studije, rezultati su pokazali da su se u SP grupi u odnosu na BP grupu, statistički značajno češće javljali acidoza (37,8% : 2,5%) i bazni eksces (35,1% : 7,5%), a zabeležene su i niže vrednosti koncentracije bikarbonatnih jona u krvi (40,5% : 20,0%) ($p < 0,001$). **Zaključak.** "*Belgrade pouch*" od kraćeg ilealnog segmenta dužine 25−35 cm omogućava odgovarajući kapacitet neobešike i smanjuje broj i učestalost metaboličkih komplikacija u poređenju sa standardnom tehnikom neobešike uz upotrebu dužeg crevnog segmenta.

Ključne reči:
mokraćna bešika, neoplazme; hirurgija, urološka, procedure; creva, rezervoari; srbija; metabolizam.

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Introduction

Longer than one century, urological surgeons are trying to find an ideal substitute for the urinary bladder in patients who require cystectomy [1]. Radical cystectomy still represents a golden standard for the treatment of muscle-invasive, organconfined bladder cancer in clinical stages T2−T4a, N0−Nx [2]. The creation of the new urinary bladder, so-called neobladder, or urinary pouch, should meet the following criteria: a neobladder should be a low-pressure reservoir, with acceptable volume and relatively small resorptive surface. The large inner surface of a pouch is associated with the high resorption of urine metabolites through intestinal mucosa and systemic metabolic disturbances [3].

There are over 40 different types of orthotopic neobladders; however, the most frequently created are neobladders made from 45−70 cm long segment of the terminal ileum [4, 5]. Unfortunately, during the time, the capacity of the neobladder increases, even up to eight times, which is followed by the increase of the resorptive surface of the pouch [6–8]. There are several consequences of that phenomenon: poor emptying of the reservoir, deterioration of the upper urinary tract, and metabolic complications. Metabolic complications are the most frequent late complications of the neobladder formation, so, every procedure which decreases their frequency and severity is of major importance.

Nowadays the management of patients with malignancy should offer not only the most curable oncological procedure, but a high level of quality of life whenever it is possible. The "Belgrade pouch" is a small step in that direction.

Methods

This prospective clinical study included 77 patients who had underwent radical cystectomy and orthotopic neobladder, from 2003 to 2012 in the Military Medical Academy, Clinic of Urology in Belgrade, Serbia. All the patients had muscle-invasive transitional cell carcinoma (TCC) of the bladder. This study was a part of the larger research ongoing project "The modification of orthotopic intestinal urine reservoir after radical cystectomy". The patients were divided into two groups. The first one was the standard pouch (SP) group of 37 patients with cystectomy and standard orthotopic neobladder, made from 50−70 cm long terminal ileum segment. The techniques used in this group were described and named by their authors (Hautmann, Camey II, Studer). The second group was the "Belgrade-pouch" (BP) group of 40 patients with cystectomy and the original, modified orthotopic neobladder, created from 25−35 cm long terminal ileum segment, in the form of the letter "U". Minimal mesenterial incision and direct ureterointestinal anastomosis were performed. Uretero-pouch anastomosis was performed with 6 (5−7) stitches, and traction of 0.5 kg on the urinary catheter was administrated for two days. Twice a day we flashed neobladder with 20 mL 0.9% NaCl solution until the catheter was removed.

All the patients underwent the measurement of neobladder capacity and arterial blood pH, base excess and bicarbonate concentration. Measurements were repeated in the postoperative months 3, 6, 12 and 15.

Patients with disease progression, diabetes mellitus, chronic obstructive pulmonary disease and patients receiving postoperative chemotherapy were excluded from the study. All the patients underwent the following arterial blood analyses: pH level, base excess (BE) and bicarbonate ion concentration (HCO_3^-) preoperatively and in postoperative months 3, 6, 12 and 15. The values of the parameters from the arterial blood that were considered abnormal were: pH < 7.35, BE > 2 and $HCO3^-$ < 22 mmol/L.

For the minimal number of examinees *per* group (24 patients) and coefficient $\alpha = 0.05$, the power of the study (1-β) was over 80%.

Results

The patients from the SP group had a significantly higher neobladder capacity than the patients from the BP group, during the whole follow-up ($p < 0.001$). In addition, at the end of the study, the patients from the SP group had much higher neobladder capacity than the natural bladder (750 mL, range: 514−2050 mL), while the patients from the BP group had the average capacity of 438 mL (range: 205−653 mL). At the end of the study, there were more patients with acidosis (37.8% : 2.5%), base excess (35.1% : 7.5%) and low bicarbonate level (40.5% : 20.0%) in the SP group than in the BP group, respectively ($p < 0.001$) (Figures 1 and 2).

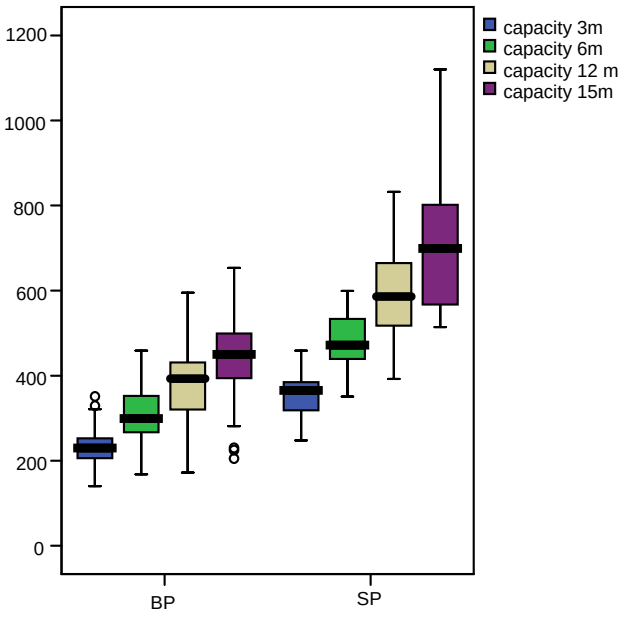

Fig. 1 – Increasing of pouch capacity in the "Belgrade pouch" (BP) and standard pouch (SP) groups. (m – postoperative month).

Variance analysis confirmed significantly different values for neobladder volume in the groups SP and BP ($F = 113.612$; $p < 0.001$; $Eta^2 = 0.609$). The neobladder capacity increased significantly over time in both groups. The

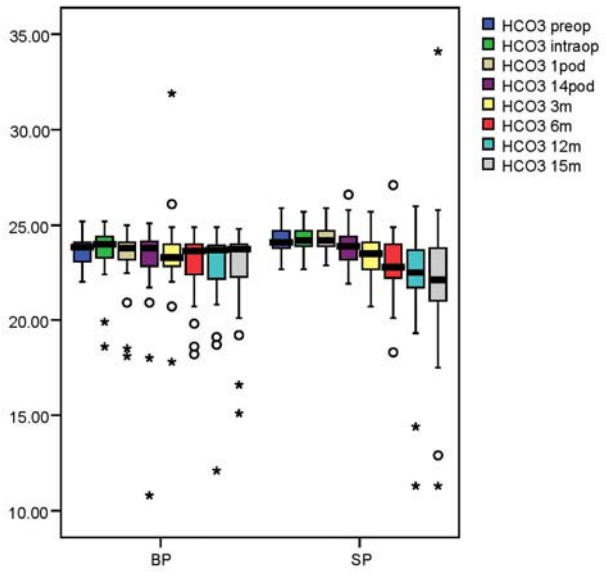

Fig. 2 – Decreasing of HCO_3^- concentration in the "Belgrade pouch" (BP) and standard pouch (SP) group during the time. preop – preoperatively; intraop – intraoperatively; pod – postoperative day; m – postoperative month.

length of the intestinal segment had a very significant influence on the change of the neobladder capacity ($F = 31.840$; $p = 0.000$; $Eta^2 = 0.304$). The results are shown in Table 1.

There were statistically significant differences between the two groups in the change of pH ($F = 15.428$; $p = 0.000$; $Eta^2 = 0.171$), the values of BE ($\chi^2 = 4.439$; df = 1; $p = 0.035$) and the change in HCO_3^- concentration ($F = 2.791$; $p = 0.045$; $Eta^2 = 0.036$). However, the most significant differences appeared after the postoperative month 15 (Figure 3).

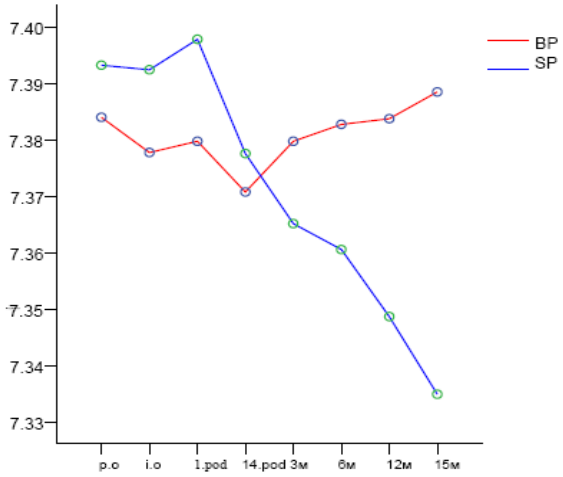

Fig. 3 – Level of acidosis in the "Belgrade pouch" (BP) and standard pouch (SP) groups; po – preoperatively; io – intraoperatively; m – postoperative month; pod – postoperative day. There was no significant change of pH in the BP group at the end of the study.

Table 1

Metabolic parameters of the BP and SP groups

Parameter	Time	BP	SP	*p*	Statistics
Male gender, (% of patients)	-	82.5	88.9	0.429	$\chi^2 = 0.625$; df = 1
Age (yeras), mean (range)	-	61.66 (42–47)	59.11 (40–73)	0.202	t = 1.288; df = 72;
Lenght of terminal ileum (cm), mean (range)	-	28.9 (20–35)	57.5 (50–70)	< 0.001**	Mann Whitney Z = -7.588
Neobladder capacity (mL), mean (range)	3 m	237 (150–351)	358 (248–459)	< 0.001**	Variance analysis
	6 m	312 (168–459)	486 (351–599)	< 0.001**	
	12 m	372 (172–558)	598 (392–832)	< 0.001**	
	15 m	438 (205–653)	750 (514–2050)	< 0.001**	
Acidosis (% of patients)	14 pod	10	0	0.144	$\chi^2 = 2.136$; df = 1
	3 m	0	10.8	0.105	$\chi^2 = 2.630$; df = 1
	6 m	2.5	10.8	0.310	$\chi^2 = 1.032$; df = 1
	12 m	2.5	32.5	0.001**	$\chi^2 = 12.272$; df = 1
	15 m	2.5	37.8	0.001**	$\chi^2 = 15.302$; df = 1
BE (% of patients)	14 pod	15.0	8.1	0.558	$\chi^2 = 0.343$; df = 1
	3 m	15.0	21.6	0.452	$\chi^2 = 0.567$; df = 1
	6 m	12.5	24.3	0.179	$\chi^2 = 1.806$; df = 1
	12 m	12.5	32.4	0.035	$\chi^2 = 4.439$; df =1
	15 m	7.5	35.1	0.003	$\chi^2 = 8.917$; df = 1
Decrease in the level of bicarbonats (% of patients)	14 pod	10.0	2.7	0.403	$\chi^2 = 0.698$; df = 1
	3 m	5.0	16.2	0.216	$\chi^2 = 1.532$; df = 1
	6 m	12.5	13.5	> 0.05	$\chi^2 = 1.122$; df = 1
	12 m	15.0	29.7	0.119	$\chi^2 = 2.424$; df = 1
	15 m	20.0	40.5	0.048*	$\chi^2 = 3.871$; df = 1

BP – Belgrade pouch; SP – standard dimensioned pouch; BE – base excess; pod – postoperative day; m – postoperative month; *statistically significant difference; **statistically highly significant difference.

Discussion

Some of the pioneers in the field of orthotopic neobladder, like Hautmann et al. [9], advocated the use of 60−70 cm long segment of the terminal ileum, for the creation of the reservoir. They considered that the neobladder required sufficient capacity for the acceptable urine continence. In contrast, some of the most important European urologists, like Hautmann et al. [9] and Studer et al. [10], advocate the use of shorter intestinal segments, in order to avoid unnecessary large capacity and numerous consequent metabolic disorders. They found that the best neobladder capacity was about 450 mL [11]. In addition, Sevin et al. [11] and Constantinides et al. [12] published acceptable functional results, using short, 36−40 cm long terminal ileal segment.

In our study, the average length of the terminal ileal segment was 28.9 cm. Like other studies, our study proved that the capacity of standard neobladders overcame the capacity of the natural urinary bladder after the postoperative month 12. However, "Belgrade-pouch" neobladders reached natural bladder capacity from postoperative month 9 to 12. In the BP group, the graduate increase of the capacity was slow, providing a high level of continence, which was previously described. In previous papers we reported that "Belgrade pouch" obtains clinically insignificant postvoided residual urine, no significant hydronephrosis due to uretero-pouch reflux, and reasonable frequency [13, 14].

A high percent of patients with classical neobladder, 25−50%, have acidosis [15]. Some authors believe that this percent could be lower if the intestinal segment was shorter than 45 cm [16, 17]. The authors of this work think that the length of the ileal segment should have been even shorter, about 30 cm, to provide the low rate of metabolic complications, with the acceptable capacity and continence.

Usually, patients with acidosis experience a long period without the symptoms; however, after that, they are prone to serious, even life-threatening complications. In 6% of cases, acute metabolic disorders require the treatment in the intensive care unit [15, 16]. In our study, there was a modest decrease in pH value on the postoperative day 14: it could be explained by the fact that it was the time when urinary catheter is usually removed. After the catheter removal, the walls of the pouch stretch and the urine comes in longer contact with intestinal mucosa. After this period, acidosis was noted in only 2.5% of patients in the BP group, while in the SP group that percent was constantly increasing, parallel to the increase of the neobladder capacity. The average pH value in the BP group was inside the normal range (7.35−7.45), while in the SP group, the average pH value was lower than 7.35 after the month 12.

Base excess is a good indicator of metabolic acidosis or alkalosis [17]. In the SP group, the percentage of patients with absolute BE > 2 increased constantly; in the BP group, significantly smaller percentage of patients had BE out of normal range. In both groups, the average BE values were constantly increasing with time; however, the patients from the SP group had significantly higher average BE values than the patients in BP group, after the month 3 of follow-up. The average BE values also confirm that neobladders created from shorter ileal segments are better substitute for the natural bladder.

At last, the number of patients with bicarbonate concentration lower than 22 mmol/L was constantly increasing in both groups. However, the statistical difference between the groups appeared only after the month 15 of follow-up. The average bicarbonate concentration was decreasing faster in the SP group, than in the BP group.

The results from our study, concerning BE values and HCO_3^- concentration after orthotopic neobladder formation, were in concordance with other published papers [17]. However, only few authors were dealing with this problem.

Conclusion

"Belgrade-pouch", orthotopic neobladder created by the original technique with a very short ileal segment, is characterized by smaller capacity and resorptive surface than standard dimensioned pouch. It provides better quality of life and lessens the percentage of patients suffering from acidosis, base excess and bicarbonate deficiency than standard neobladder.

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Isokinetic profile of subjects with the ruptured anterior cruciated ligament

Izokinetički profil osoba sa rupturom prednjeg ukrštenog ligamenta

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Abstract

Background/Aim. All changes in the knee that appear after anterior cruciate ligament (ACL) lesion lead to difficulties in walking, running, jumping especially during sudden changes of the line of movement. This significantly impairs quality of life of these subjects and leads to decrease in physical activity. Knee injuries make 5% of all most severe acute sport injuries. The aim of the study was to determine strength of the thigh muscles in persons with unilateral rupture of the ACL and to evaluate potential bilateral differences between healthy and injured leg. **Methods.** This study involved 114 male athletes of different sport specialities with the clinical diagnosis of ACL rupture. Each subject had unilateral ACL rupture and the other leg was actually the control for this research. An isokinetic device was used to evaluate the muscle strength of thigh muscles. Testing was performed for two testing speeds, 60º/s and 180º/s. **Results.** Data analysis showed a statistically significant difference ($p < 0.01$) between the ACL and the healthy leg in the following parameters: peak torque for thigh extensors (Ptrq_E), angle to peak torque during extension (Ang_E), power of extension (Pow_E) and work during extension (Work_E). Analysing hamstrings to quadriceps (H/Q) ratio we found the unilateral disbalance of thigh muscle strength in ACL leg. **Conclusion.** A high level of validity makes isokinetic dynamometry the method for evaluation of thigh muscles strength and leaves this field of research open for new studies in order to improve both diagnostic and rehabilitation of patients with the insufficient ACL.

Key words:
knee injuries; anterior cruciate ligament; muscle, sceletal; muscle strenght.

Apstrakt

Uvod/Cilj. Promene u zglobu kolena koje nastupaju nakon lezije prednjeg ukrštenog ligamenta (*ligamentum cruciatum anterius* – LCA) dovode do poteškoća u hodanju, trčanju i skakanju, pogotovo prilikom nagle promene pravca kretanja. Ovakva pojava u značajnoj meri umanjuje kvalitet života kod takve osobe i dovodi do umanjenja nivoa fizičke aktivnosti. Povrede kolenog zgloba čine 5% ukupnog broja najtežih akutnih sportskih povreda. Cilj ovog istraživanja bio je da se utvrdi snaga mišića natkolenice kod osoba sa jednostranom rupturom LCA, kao i razlika snage mišića između zdrave i povređene noge. **Metode.** U istraživanje je bilo uključeno 114 muškaraca, aktivnih sportista, različitih sportskih disciplina, sa kliničkom dijagnozom rupture LCA. Kod svakog ispitanika postojala je ruptura LCA na jednom kolenu, tako da je zdrava noga predstavljala kontrolnu grupu u ovom istraživanju. Pomoću izokinetičkog aparata merili smo snagu mišića natkolenice i to za dve test brzine, 60º/s and 180º/s. **Rezultati.** Utvrđena je statistički značajna razlika ($p <$ 0,01) između zdrave i povređene noge i to za merene parametre: maksimalni obrtni moment za bedrene ekstenzore (Ptrq_E), maksimalni iznos ugla ekstenzije (Ang_E), snaga ekstenzije (Pow_E) i rad tokom ekstenzije (Work_E). Za odnos mišići zadnje lože/kvadriceps (H/Q) utvrdili smo da postoji izražen disbalans u mišićnoj snazi kod povređene noge. **Zaključak.** Postoji visok stepen vrednosti izokinetičke dinamometrije kao metode za određivanje snage mišića natkolenice. Ovu oblast potrebno je dodatno istraživati u cilju unapređenja kako dijagnostike, tako i rehabilitacionih programa kod osoba sa insuficijencijom LCA.

Ključne reči:
koleno, povrede; ligament, prednji ukršteni; mišići, skeletni; mišići, snaga.

Introduction

The knee is one of the biggest joints in the human body. It is a complex structure both anatomically and biomechanically. All changes in the knee that appear after the lesion of the anterior cruciate ligament (ACL) lead to difficulties in walking, running, jumping, especially during sudden changes in line of movement. This significantly impairs quality of life of these sub-

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jects and leads to decrease in physical activity. Knee injuries make 5% of all most severe acute sport injuries [1]. Anatomy and physiology of the knee dictate the type, character and degree of the consequences to the physical activity after injury. Ones of the most often and the most difficult are the injuries of the ligaments which usually are not isolated but combined with trauma of the menisci and joint capsule. Incidence of meniscus tears together with rupture of ligaments is reported to be from 37% to 86% [2, 3]. The presence of combined lesion of menisci and cruciated ligaments will soon lead to degenerative changes of joint cartilage of knee.

Clinical examination has the leading role in putting of the proper diagnosis of the soft tissue structures of the knee together with functional tests [4].

During inspection of the injured knee we can observe swollen structures due to rupture of blood vessels which nourish the ACL, though the absence of one does not mean that it is not ruptured. In old ruptures of the ACL we can see atrophy of the medial head of quadriceps muscle (*musculus vastus medialis*) because of immobilisation and/or decrease in activity due to pain. Palpation is used to assess state of collateral ligaments. Stability evaluation of the knee joint is performed using clinical examination tests (Lachman test, Pivot shift test, tests of front and rear "drawer") which can be used in both old and acute cases. According to data from the literature, the most accurate results are obtained *via* "Lachman" test and "Pivot shift" test [5].

According to the principles of good practise it is necessary to perform radiographic imaging [anterior-posterior (AP) and profile scan] in order to exclude possible avulsion of bony part of ligament insertion or associated trauma [6]. Introduction of magnetic resonance imaging (MRI) changed the approach to evaluation of ligament lesions of the knee [7, 8]. Noninvasiveness and the absence of harmful radiation are the main contributors to the usage of this imaging method with the reliability more than 95%.

Measuring muscle strength has significance in everyday practice both in persons with physical disorders and healthy active population [9, 10]. Dynamic recording of muscle actions can be performed with specially designed machines. Isokinetic devices measure maximal strength and other aspects of physical performances (speed, power, explosiveness) [11–13].

The aim of the study was to measure the parameters of strength of the thigh muscles in persons with unilateral rupture of the ACL, as well as to evaluate the potential bilateral differences between the healthy and the injured leg.

Methods

Our study involved 114 male athletes of different sport specialities (in average high 184.14 cm with body mass, 86.5 kg, aged 24.32 year) with the clinical diagnosis of ACL rupture. This diagnose was later confirmed during surgical reconstruction of the teared ACL. Each subject had unilateral ACL rupture and the other leg was actually the control for this research. All measurements were conducted at the Department of Physiology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia, and at the Provincial Department for Sports, Novi Sad, Serbia. All the subjects were informed in detail on the design of the study and gave their written consent.

The isokinetic device was used to evaluate muscle strength of the thigh muscles (EASY – TECH prima doc). Prior to each testing the machine was calibrated and the range of movement (ROM) set to 90°. The dynamometer seat was set for each subject in order to bring the axis of the rotation of the knee in the center of the axis of rotation of the machine. We used tapes to stabilize subjects to the seat in order to isolate the single movement of the thigh extensors and thigh flexors. Testing was performed for two testing speeds, 60º/s and 180º/s. Complete testing was performed according to standard protocol, first the injured leg (ACL leg) and then the healthy one. Five maximal contractions were performed in the concentric–concentric mode including visual feedback.

Results

Analysis of the isokinetic parameters for the test speed of 60°/s found a statistically significant difference ($p < 0.01$) between the ACL leg and the healthy leg for following parameters: peak torque for thigh extensors (Ptrq-E), angle to peak torque during extension (Ang-E), power of extension (Pow-E) and work during extension (Work-E) (Table 1). The

Table 1

Analysis of the isokinetic parameters for the test speed of 60º/s

Parameter	Healthy leg	ACL leg	*t*	*p*
	x̄ ± SD	x̄ ± SD		
Ptrq_E (nm)	264.2 ± 30.8	213.9 ± 43.6	-5.565	0.000*
Ptrq_F (nm)	125.9 ± 19.4	119.1 ± 27.4	-1.240	0.218
Ang_E (°)	23.1 ± 8.5	27.9 ± 9.1	2.392	0.019*
Ang_F (°)	23,6 ± 5.2	25.4 ± 12.4	1.475	0.143
Pow_E (W)	377.0 ± 59.5	316.9 ± 74.0	-3.562	0.001*
Pow_ F (W)	146.5 ± 21.6	137.5 ± 37.8	-0.934	0.353
Work_E (J)	1135.4 ± 104.9	984.9 ± 156.4	-4.520	0.000*
Work_F (J)	295.7 ± 32.6	290.7 ± 48.3	-0.249	0.804

***Statistically significant difference, $p < 0.01$ (except for the parameter Ang_E for which the significance was set at $p < 0.05$.**

ACL – anterior cruciate ligament.

Ptrq_E – peak torque for thigh extensors; Ptrq_F – peak torque for thigh flexors; Ang_E – angle to peak torque during extension, Ang_F – angle to peak torque during flexion; Pow_E – power of extension; Pow_ F – power of flexion; Work_E – work during extension; Work_F work during flexion; nm – newton metre; W – watt; J – joule.

parameters peak torque for thigh flexors angle to peak torque during flexion, power of flexion and work during flexion did not show any significant difference.

For the test speed of 180°/s analysis of the isokinetic parameters revealed the same result (Table 2). Hamstrings to quadriceps (H/Q) ratio for both testing speeds presenting unilateral disbalance in ACL leg is shown in Figure 1.

Table 2

Analysis of the isokinetic parameters for the test speed of 180º/s

Parameter	Healthy leg	ACL leg	*t*	*p*
	x̄ ± SD	x̄ ± SD		
Ptrq_E (nm)	213.3 ± 40.7	178.8 ± 43.4	4.298	0.000*
Ptrq_F (nm)	130.3 ± 39.5	114.4 ± 40.4	-1.001	0.320
Ang_E (°)	17.4 ± 11.4	23.5 ± 12.7	-4.147	0.000*
Ang_F (°)	26.8 ± 25.9	27.6 ± 24.9	0.981	0.330
Pow_E (W)	716.0 ± 134.9	597.1 ± 156.7	-4.885	0.000*
Pow_ F (W)	259.7 ± 86.6	237.8 ± 90.2	-1.228	0.223
Work_E (J)	1029.7 ± 213.8	844.4 ± 236.5	5.265	0.000*
Work_F (J)	258.4 ± 82.4	230.7 ± 79.1	0.141	0.888

***Statistically significant difference ($p < 0.01$);**
For abbreviations, see under Table 1.

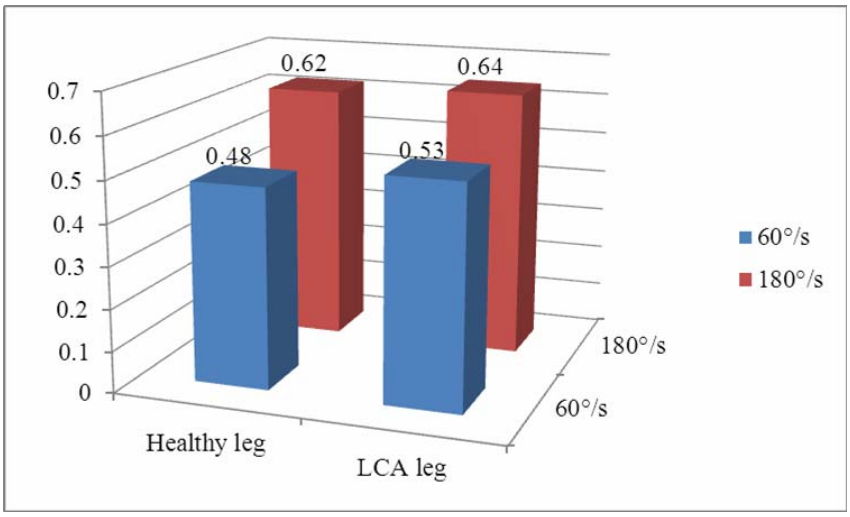

Fig. 1 – Hamstrings to quadriceps (H/Q) ratio for both testing speeds.
LCA – *ligamentum cruciatum anterius*.

Discussion

Assessment of voluntary muscle action is the key element in research of physical performance in human subjects. Gathered information are used by both researchers and clinicians to create rehabilitation or training protocols to cope with disabilities and impairments. In the late 80s of the 20th century isokinetic devices appeared on the scene of muscle testing. This device adapts to the strength of the test subject by maintaining the constant speed of movement through the whole ROM. The reduction of pain and discomfort during testing is extended greatly, so this method was possible to introduce in early stages of medical rehabilitation in patients, as well as in the population of healthy competitive sportsman.

Special attention is paid to anatomical and functional integrity of the knee, that is active and passive stabilizers. In case of rupture of ACL it is probable that active competitive carrier of an athlete is over. However, nowadays orthopaedic surgery is able to perform reconstruction of the defect which enables further active training and competition in athletes and in recreational individual avoidance of progressive degenerative changes in the knee.

A revolutionary study of Johnson [14] in the field of isokinetic rehabilitation after surgical reconstruction of ACL was presented in 1982. He explained the use of the anti-shear mechanism on the isokinetic device which provided early rehabilitation protocol to be introduced. This device protected the knee against front movement of the distal part of the lower leg and acted as a protective factor to newly reconstructed graft. In this way Johnson provided so much required early rehabilitation protocol after ACL reconstruction.

In the first 20 years of exploitation of isokinetic dynamometry the knee joint was in the center of interest. Analysing the literature we spotted that over 75% of all studies referring to isokinetics was dealing with the knee physiology, pathology and training. Nevertheless, the knee joint is still in the focus though not in such an extent. The reason could be successful surgery and rehabilitation protocols after injury [15–17].

Testing speed that the isokinetic device can provide varies from 0º/s to 550º/s. For testing muscle strength and en-

durance it is proposed to use speeds ranging from 60º/s to 180º/s [18]. In our research we used these testing speeds as proven to be most appropriate.

Analyzing the gathered data it appears that the muscle strength for the left and the right leg in the same subject does not differ more than 10%. This became the standard value of bilateral difference of muscle strength for quadriceps and hamstring muscles. Calmels et al. [19] reported that in the group of healthy high school children tested at 60º/s, 120º/s and 180º/s no significant bilateral difference was found.

Capranica et al. [20] and Brady et al. [21] found no significant difference in strength parameters recorded *via* the isokinetic dynamometer between the dominant (kicking) leg and nondominant one in the group of competitive soccer players.

Epidemiological studies following the incidence of sport injuries reported 20% of all injuries to be related with the knee [22–24]. This data is referring to all the participants in the sport both recreational and competitive. These studies also showed the incidence of injuries in relation to specific sport. Sports with the incidence close to 20% were football, basketball, soccer and show dance. A somewhat lower incidence, around 10%, was reported in boxing, fencing, ice hockey. The lowest incidence was found in cycling and swimming, less than 5%. Among the most severe injuries during sporting activities (5%) are accounted to injuries of ligaments and menisci of the knee [1].

Bilateral muscle testing is one of the most used methods for evaluation the results in isokinetics [25]. Subjects with knee pathology are at the same time both the control and the experimental group since one leg is healthy and the other one is with specific pathology. Bilateral muscle strength asymmetry more than 10% suggests the presence of changes in muscles, tendons and ligaments. Grace et al. [26] presented that the difference in extensors of the knee was 16.6% and in flexors 11%. Similar results were presented in a recent study by de Jong et al. [27] where they found the deficit of 13.6%.

In our study in the group of athletes with insufficient ACL we found deficit for extensors (16%) and flexors (9.7%) for the testing speed of 60º/s, while for the testing speed of 180º/s we found the deficit of 16% and 12%, respectively.

Dvir et al. [28] reported findings in the group of subjects with chronic lesion of ACL. Rupture was 1.5 years old on the average. Testing thigh muscles was conducted according to the standard testing protocol for the testing speed of 30°/s in the concentric-concentric mode. The results showed the average deficit of 21% and 14% for extensors and flexors, respectively. Bonamo et al. [29] followed a group of recreational athletes with the diagnosed ACL tear. They tested them for the testing speed of 60º/s and found the deficit for extensors 11% and for flexors only 3%. The subjects were treated conservatively but never were able to return to full level of performance prior to injury.

It is noticed that for the lower testing speeds the observed deficit between the injured leg and the healthy one is higher. As the speed increases the deficit is getting less obvious. A group of papers proposed explanation to this statement [30–32]. At the lower testing speed there is higher force acting to the joint capsule during movement. Since the knee is compromised due to the trauma pain appears during quadriceps contraction which provokes a significant muscle spasm of extensors and reflex contraction of thigh flexors. Such mechanism further attenuates force output of the quadriceps. Tsuda et al. [33] presented firm evidence for a connection between the reflex activity of the ligaments of the knee and thigh muscles.

The ratio between flexor and extensor muscles of the thigh is considered as good indicator of normal – physiological functionality of these muscles though it is highly dependent on testing speed [34]. For lower testing speeds (0º/s – 60º/s) it is 50–60% depending on test subjects and the isokinetic device. The H/Q ratio is especially significant in the acyclic sports and in the dominant side of the body [35, 36].

Our results for testing speeds of 60º/s and 180º/s for the ACL leg were 53 and 64 and for healthy leg 48 and 62, respectively. A possible explanation of this finding could be that there is no significant difference in the measured isokinetic strength of thigh flexors. Namely, thigh flexors act as knee stabilizers in subjects with insufficient ACL. They prevent forward movement of tibia during forceful extensor actions. Aside to this strictly numerical analysis researchers noticed that the curve obtained during the recording of the movement on isokinetic device can be very informative [37]. Beside curve analysis, electromyography recording is a useful tool, as well. In this way voluntary muscle action can be monitored for the level of effort and even become a legal medical tool for assessment of impairment [38].

Conclusion

Isokinetic testing of thigh muscles provides solid parameters which define functional abilities of the tested muscles. Our results present a statistically significant difference between the ACL and the healthy legs for the following parameters: Ptrq_E, Ang_E, Pow_E and Work_E. Analysing the H/Q ratio showed a unilateral disbalance in the ACL leg.

The gathered results indicate a high level of validity of isokinetic dynamometry as the method for evaluation of thigh muscles strength and leave this field of research open for new studies in order to improve both diagnostic and rehabilitation of patients with the insufficient ACL.

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ORIGINAL ARTICLE

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In vitro assessment of antiproliferative action selectivity of dietary isothiocyanates for tumor *versus* normal human cells

In vitro ispitivanje selektivnosti antiproliferativnog dejstva dijetetskih izotiocijanata na tumorske u odnosu na normalne humane ćelije

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Abstract

Background/Aim. Numerous epidemiological studies have shown beneficial effects of cruciferous vegetables consumption in cancer chemoprevention. Biologically active compounds of different *Brassicaceae* species with antitumor potential are isothiocyanates, present in the form of their precursors – glucosinolates. The aim of this study was to determine the selectivity of antiproliferative action of dietary isothiocyanates for malignant *versus* normal cells. **Methods.** Antiproliferative activity of three isothiocyanates abundant in human diet: sulforaphane, benzyl isothiocyanate (BITC) and phenylethyl isothiocyanate, on human cervix carcinoma cell line – HeLa, melanoma cell line – Fem-x, and colon cancer cell line – LS 174, and on peripheral blood mononuclear cells (PBMC), with or without mitogen, were determined by MTT colorimetric assay 72 h after their continuous action. **Results.** All investigated isothiocyanates inhibited the proliferation of HeLa, Fem-x and LS 174 cells. On all cell lines treated, BITC was the most potent inhibitor of cell proliferation with half-maximum inhibitory concentration (IC_{50}) values of 5.04 mmoL m^{-3} on HeLa cells, 2.76 mmol m^{-3} on Fem-x, and 14.30 mmol m^{-3} on LS 174 cells. Antiproliferative effects on human PBMC were with higher IC_{50} than on malignant cells. Indexes of selectivity, calculated as a ratio between IC_{50} values obtained on PBMC and malignant cells, were between 1.12 and 16.57, with the highest values obtained for the action of BITC on melanoma Fem-x cells. **Conclusion.** Based on its antiproliferative effects on malignant cells, as well as the selectivity of the action to malignant *vs* normal cells, benzyl isothiocyanate can be considered as a promising candidate in cancer chemoprevention. In general, the safety of investigated compounds, in addition to their antitumor potential, should be considered as an important criterion in cancer chemoprevention. Screening of selectivity is a plausible approach to the evaluation of safety of both natural isothiocyanates and synthesised analogues of these bioactive compounds.

Key words:
isothiocyanates; vegetables; neoplastic cells, circulating; lymphocytes; chemoprevention.

Apstrakt

Uvod/Cilj. Brojne epidemiološke studije pokazale su povoljne efekte konzumiranja povrća iz familije kupusnjača (*Brassicaceae*) u hemioprevenciji karcinoma. Osnovni biološki aktivni sastojci ovog povrća su izotiocijanati, prisutni u obliku prekursora – glukozinolata. Cilj ovog rada bio je određivanje selektivnosti antiproliferativnog delovanja dijetetskih izotiocijanata na maligne ćelije u odnosu na normalne ćelije. **Metode.** Antiproliferativna aktivnost tri izotiocijanata zastupljena u ljudskoj ishrani: sulforafana (SFN), benzil-izotiocijanata (BITC) i feniletil-izotiocijanata (FEITC) na humane maligne ćelijske linije, HeLa, ćelijsku liniju karcinoma grlića materice, Fem-x, ćelijsku liniju melanoma, i LS 174, ćelijsku liniju karcinoma kolona, kao i na mononuklearne ćelije periferne krvi (MNĆPK), sa ili bez delovanja mitogena, određivana je MTT kalorimetrijskim testom, 72 h nakon kontinuiranog delovanja agenasa. **Rezultati.** Svi ispitivani izotiocijanati inhibirali su proliferaciju HeLa, Fem-x i LS 174 ćelija. Na svim ćelijskim linijama BITC je pokazao najizraženije delovanje sa vrednostima polumaksimalne inhibitorne koncentracije (IC_{50}) od 5.04 mmol m^{-3} na HeLa ćelijama, 2,76 mmoL m^{-3} na Fem-x i 14,30 mmol m^{-3} na LS 174 ćelijama. Svi ispitivani izotiocijanati pokazali su citotoksično delovanje na MNĆPK, ali sa višim IC50 vrednostima u odnosu na maligne ćelije. Indeksi selektivnosti antitumorkog delovanja, izraženi kao odnos IC_{50} vrednosti dobijenih na MNĆPK i malignim

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ćelijama, bili su između 1,12 i 16,57, sa najvišom vrednosti pri delovanju BITC na Fem-x ćelije. **Zaključak.** Na osnovu antiproliferativne aktivnosti na maligne ćelije i selektivnosti antiproliferativnog delovanja na maligne u odnosu na normalne ćelije, benzil izotiocijanat se ističe kao perspektivni agens u hemioprevenciji karcinoma. Generalno, pored antitumorskog delovanja, bezbednost primene ovih jedinjenja treba da predstavlja važan kriterijum u izboru odgovarajućih izotiocijanata za primenu u primarnoj, sekundarnoj i tercijarnoj hemioprevenciji kancera. Ispitivanje selektivnosti predstavlja pogodan pristup oceni bezbednosti i prirodnih izotiocijanata i sintetskih analoga.

Ključne reči:
izotiocijanati; povrće; neoplazme, cirkulišuće ćelije; limfociti; hemoprevencija.

Introduction

Numerous epidemiological studies have shown an inverse association between cruciferous vegetable intake and risk of different types of cancer [1–5]. Cruciferous vegetables, including broccoli, cauliflower, cabbage, kale, Brussels sprouts, water cress, and rocket, among others, are rich sources of sulphur containing compounds – glucosinolates (GLs). Basic structure of GLs consists of a β-D-thioglucose group, a sulfonated oxime group and a side chain derived from different amino acids. The most abundant source of these plant constituents is the family *Brassicaceae* (*Cruciferae)* in which they were discovered [6]. Glucosinolates undergo hydrolysis by endogenous enzyme myrosinase, to yield glucose, sulphuric acid and a molecule of thiocyanates, nitriles, indoles or isothiocyanates (ITCs) depending on the side chain in parent glucosinolate [7]. Myrosinase accompanies the GLs in the plant tissue, placed in cells separately from GLs and released only after the degradation of cell walls (by chopping, chewing), thus catalysing the generation of isotiocyanates. Intestinal flora also possesses myrosinase activity [7].

Numerous studies, both *in vitro* and *in vivo*, have indicated that the beneficial effects of cruciferous vegetables in cancer prevention are the result of the action of ITCs rather than the action of their precursors – glucosinolates [8, 9]. Mechanisms of anticancer activity of ITCs are numerous [10]. They act both as blocking and suppressing agents able to impede initiation, promotion and/or progression of carcinogenesis [11]. Inhibition of carcinogenesis during the initiation stage is presumably associated with the modulation of carcinogen metabolism, including inhibition of metabolic activation of carcinogens by phase I enzymes, coupled with induction of detoxifying phase II enzymes [12]. Inhibition of tumor cell proliferation is crucial for the inhibitory effects of ITCs on promotion and progression of carcinogenesis [13]. Other chemopreventive mechanisms include induction of apoptosis, prevention of neoangiogenesis, anti-migratory or epigenetic effects [8, 14]. Scientific evidence of antitumor potential of ITCs provides a rationale for their use as chemopreventive agents [15, 16]. Structural differences are the result of different side group in a relatively simple chemical structure of ITCs and determine specific mechanistic profile of individual molecules regarding both antitumor action and their safety [17]. Selectivity of ITCs action towards malignant cells is the main criterion for their potential use in primary, secondary or tertiary prevention. In general, the balance between efficacy and safety (in addition to the costs and practicality of the intervention) is crucial criterion in the evaluation of chemopreventive agents regardless the nature and mechanism of their action. The balance is shifted to the safety for agents with the main role in primary and secondary prevention, while in tertiary prevention and therapy efficacy takes the priority [18].

Numerous data have shown that even subtle change in chemical structure of the ITCs can have a profound effect on their activity and mechanism of action, which have opened a wide field of chemical synthesis of ITC analogues with targeted effects and increased efficacy [19–23]. Evaluation of their chemopreventive potential in terms of fine balance between efficacy and safety is an important issue for their further use in practice.

Sulforaphane (SFN), benzyl isothiocyanate (BITC) and phenyl ethyl isothiocyanate (PEITC) are ITCs often present in human diet (based on the intake of food rich in their precursor GLs). Cruciferous vegetables usually contain a large number of structurally different GLs. However, SFN, in the form of corresponding GL - glucoraphanin, is the major bioactive compound in broccoli, BITC in the form of glucotropaeolin, is the major bioactive in garden cress, and PEITC in the form of GL – gluconasturtiin, is major bioactive of watercress [24].

The aim of this study was to provide data on antiproliferative action of SFN, BITC and PEITC on a panel of malignant cell lines in the presence of human sera as a model closer to in *vivo* conditions and with specific focus on the selectivity of their antitumor action. Selectivity of antiproliferative action towards malignant cells was determined by simultaneous investigation of their effects on peripheral blood mononuclear cells (PBMC) of healthy volunteers. Applied experiments are proposed to be used as a plausible and practical screening model in the preclinical evaluation of cancer chemopreventive profile of natural isothiocyanates and synthesised analogues of these bioactive compounds, including both safety and efficacy.

Methods

Cell cultures and chemicals

Human cervix carcinoma cell line (HeLa), human melanoma cell line (Fem-x) and human colon cancer cell line (LS 174) used in the study were obtained from the American Type Culture Collection (Manassas, VA, USA). Cell lines were cultured as monolayers in the nutrient medium, i.e. RPMI 1640 medium (Sigma–Aldrich, Germany) supplemented with L-glutamine (3 moL m^{-3}), streptomycin (100 mg L^{-1}), penicillin (100 IU mL^{-1}) and grown at 37°C in atmosphere with 5% CO_2, 95% air and 95% relative humidity.

Stock solutions of ITCs (Sigma–Aldrich, Germany) were made in dimetyl sulfoxide (DMSO) at concentrations of 28.3 moL

m^{-3} for SFN, 75.4 moL m^{-3} for BITC and 66.8 moL m^{-3} for PEITC, filtered through Milipore filter 0.22 μm and kept at – 20°C.

The whole blood for isolation of sera and PBMC was obtained from healthy subjects according to the protocol approved by Ethical Committee of the Institute for Oncology and Radiology of Serbia (within the project No 175011). The study was undertaken according to the Helsinki Declaration and all the subjects gave written informed consent prior to the enrolment. Human AB+ sera isolated from the whole blood of healthy volunteers was pooled and used further according to the protocol.

Preparation of peripheral blood mononuclear cells

PBMC were separated from the whole heparinized blood of healthy volunteers by gradient centrifugation (Lymphoprep™, Norway). Cells collected from the interface were washed three times with Haemaccel® (aqueous solution supplemented with 145 mol m^{-3} Na^+, 5.1 moL m^{-3} K^+, 6.2 moL m^{-3} Ca^{2+}, 145 moL m^{-3} Cl^- and 35 g L^{-1} gelatin polymers, pH = 7.4), counted and re-suspended in nutrient medium.

Treatment of cell lines

HeLa and Fem-X cells were seeded at a density of 2,000 cells *per* well in 96-well plates, in nutrient medium supplemented with human serum (a mass fraction of 10%) in total volume of 100 μL. LS 174 cells were set up at a density of 7,000 cells *per* well and grown similarly.

The following day, after the adherence, cells were treated with ITCs. Briefly, stock solutions were diluted in nutrient medium supplemented with fresh AB+ human serum (a mass fraction of 10%) and 50 μL of the obtained working solutions was added to the wells. Final concentrations of ITCs tested for antiproliferative action on cancer cell lines were 1, 5, 10, 25, 50 μM. Concentration of DMSO in the cell did not exceed 0.17%. Wells with cells treated with DMSO solution in nutrient medium, in concentrations equal to the DMSO level in wells with 50 μM ITCs (i.e. 0.17% in experiments with SFN, 0.07% with PEITC and 0.075 % with BITC) were used as controls. It was shown in preliminary experiments that in these concentrations DMSO do not influence the proliferation and the survival of the malignant or normal cells. Wells containing ITCs in investigated concentrations but void of cells were used as corresponding blanks.

Treatment of peripheral blood mononuclear cells

PBMC were seeded in 96-well plates (150,000 cells *per* well) in nutrient medium supplemented with 10% autologous human serum, or in supplemented nutrient medium enriched with phytohaemaglutinin (PHA; 5 mg L^{-1}; Sigma-Aldrich, Germany). Stock solutions of ITCs were diluted in nutrient medium supplemented with autologous serum (a mass fraction of 10%) and working solutions was added to the wells. Final concentrations of ITCs tested for their action on PBMC were 5, 10, 20, 40 and 80 μM. Wells with PBMC and DMSO solution in nutrient medium (up to 0.17%) were used as controls and wells containing ITCs in investigated concentrations but void of cells were used as corresponding blanks. The ranges of ITCs concentrations tested (0–50 μM for experiments on cancer cell lines and 0–80 μM on PBMC) were defined based on the preliminary experiments with the highest concentration selected as the one that induce the decrease in cell survival to the 10% of the cell number (i.e. absorbance) in the control wells.

Determination of cell survival

Cell survival was assayed 72 h after the continuous ITC action, using MTT test [25]. Briefly, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Sigma-Aldrich, Germany solution (5 μg L^{-1} in phosphate buffered saline; pH 7,2) was added to each well. Samples were then incubated for four hours at 37°C in 5% CO_2 and humidified air atmosphere. Afterwards, 100 μL of 10% sodium dodecyl sulfate was added to the wells and the plates were kept overnight in the CO_2 incubator in humidified atmosphere, followed by absorbance measurements. The absorbance (A) was measured at 570 nm. The cell survival (%) was calculated by division of A of sample with cells grown in the presence of various concentrations of investigated compounds, with control absorbance (Ac) of cells grown only in nutrient medium, and multiplied with 100. It was implied that A of blank was always subtracted from A of corresponding sample with target cells. The half-maximal inhibitory concentration (IC_{50}) was determined as a concentration of a compound that inhibited cell proliferation by 50% compared to control wells; i.e. that resulted in cell survival of 50%.

Statistical analysis

All results were presented as mean ± standard deviation (SD) of five independent experiments. All experiments are performed in triplicates. IC_{50} values were extracted from dose-response curves for each ITC on each cell type, as a concentration of a drug that inhibited cell survival by 50%.

Results

The results obtained for antiproliferative action of investigated compounds show that all investigated ITCs, SFN, BITC, and PEITC, significantly inhibited proliferation of cultured HeLa, Fem-x and LS 174 cells. Based on IC_{50} values (Table 1) BITC was the most potent inhibitor of cell proliferation in all cell lines treated. The potencies of SFN and PEITC were similar. The order of sensitivity of various human cancer cell lines to the antiproliferative action of ITCs in descending order was: human melanoma, Fem-x cells > human cervix adenocarcinoma, HeLa cells > human colon carcinoma, LS 174 cells.

The obtained results show that BITC has the most potent antiproliferative action compared to SFN and PEITC in HeLa and LS 174 cells. Our data from this work also show antiproliferative potency of tested ITC also on melanoma Fem–

Table 1

Half-maximal inhibitory concentration (IC_{50}) for the antiproliferative action of the investigated compounds on malignant and normal human cells determined by MTT assay after 72 h of continuous action

Cell line/compound	IC_{50} (mmoL m^{-3})		
	SFN	BITC	PEITC
HeLa	13.59 ± 1.53	5.04 ± 1.73	12.00 ± 1.97
Fem-x	6.67 ± 0.73	2.76 ± 0.58	6.22 ± 0.32
LS 174	16.09 ± 2.44	14.30 ± 5.16	18.23 ± 3.04
PBMC	29.34 ± 15.3	45.74 ± 18.9	23.41 ± 7.13
PBMC+PHA	15.30 ± 5.39	21.92 ± 4.63	30.87 ± 6.33

Data are presented as mean ± standard deviations (SD) of five independent experiments. SFN – sulforaphane; BITC – benzyl isothiocyanate; PEITC – phenyl ethyl isothiocyanate; PBMC – peripheral blood mononuclear cells; PBMC+PHA – PBMC treated with phytohaemagglutinin; HeLa – human cervix carcinoma cell line; Fem-x – human melanoma cell line; LS-174 – human colon cancer cell line; MTT – 3-(4, 5-dimethylthiazol-2-yl) – 2,5 diphenyltetrazolium bromide.

x cell line. It should be noted that this cell line appears to be the most sensitive one. IC_{50} values for the action of SFN and PEITC on Fem-x cells were similar. It should be emphasized that both values are similar to the IC_{50} value of (5.51 ± 0.3 mmoL m^{-3}) for the action of cisplatin on Fem-x cells that was reported by Pantelić et al. [26] in the same experimental design. Even stronger antiproliferative potential of BITC on Fem-x cells compared to the action of cisplatin, with twice as lower IC_{50} value was demonstrated in the present study.

As seen in Figure 1, the shape of dose-response curves for the action of ITCs on malignant cells and PBMCs is different. A characteristic plateau could be observed in the cur-

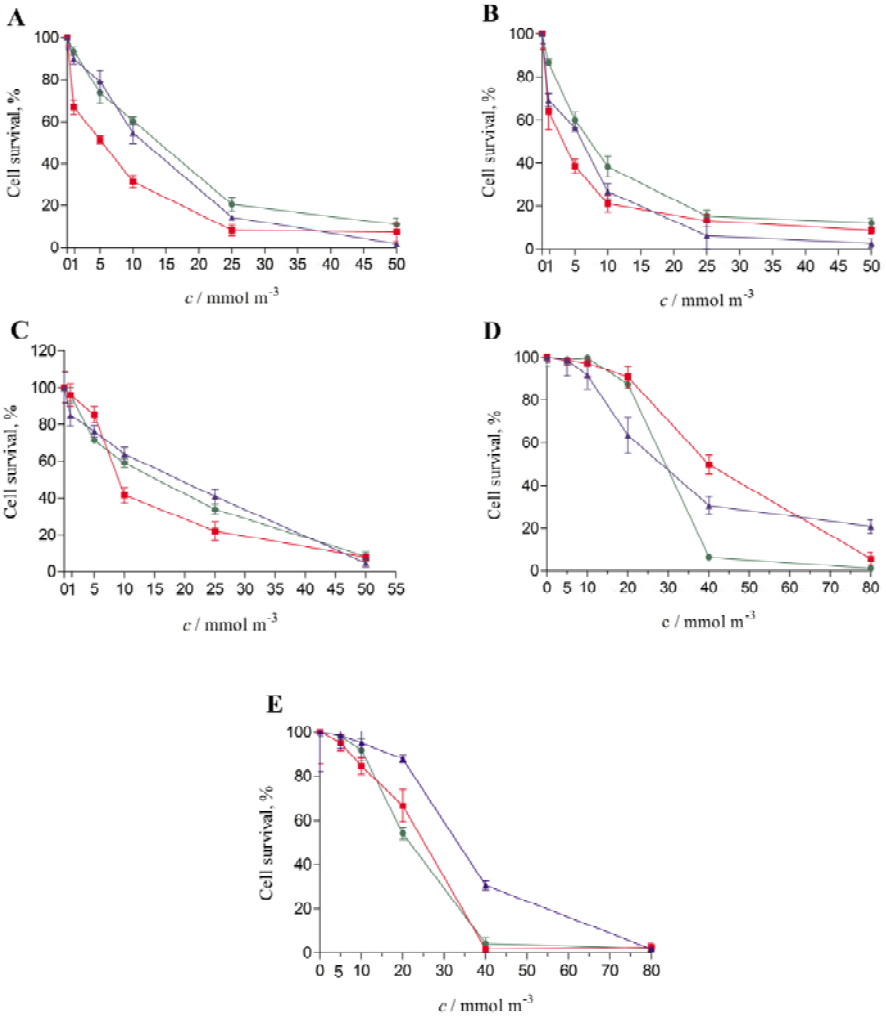

Fig. 1 – Representative graphs showing the survival of HeLa (A), Fem-x (B), LS 174 cells (C), peripheral blood mononuclear cells (PBMC, D) and PBMC stimulated with phytohaemagglutinin (E), plotted as a function of concentration (c) of SFN (●), BITC (■), and PEITC (▲), determined by MTT test 72 h after the continuous agents action. For other abbrevations see under Table 1.

ve for ITCs treated PBMC and PBMC with mitogen in the concentration range 0–5 mmoL m^{-3}, and a linear dose-response correlation with higher doses. Regarding the effects on malignant cells, linearity in the response could be observed within the whole concentration range.

The selectivity in the antitumor action was evaluated for each ITCs and was expressed as selectivity index (SI), calculated as the ratio between IC_{50} values obtained with PBMC or mitogen-treated PBMC and IC_{50} values for investigated ITCs on malignant cells. The values obtained for SI are presented in Table 2.

Table 2

Selectivity indexes (SI) of antiproliferative action of the investigated isothiocyanates

Cell lines/compound	SI*		
	SFN	BITC	PEITC
IC_{50} PBMC / IC_{50} HeLa	2.16	9.07	1.95
IC_{50} PBMC+PHA / IC_{50} HeLa	1.12	4.35	2.57
IC_{50} PBMC / IC_{50} Fem-x	4.39	16.57	3.76
IC_{50} PBMC+PHA / IC_{50} Fem-x	2.28	7.94	4.96
IC_{50} PBMC / IC_{50} LS 174	2.16	3.19	1.28
IC_{50} PBMC+PHA / IC_{50} LS 174	1.12	1.53	1.69

***SI towards malignant cell line are calculated as the ratio of the half-maximal inhibitory concentration (IC_{50}) value for PBMC (or PBMC+PHA) and IC_{50} value for corresponding cell line.**

SFN – sulforaphane; BITC-benzyl isothiocyanate; PEITC – phenyl ethyl isothiocyanate; PBMC – peripheral blood mononuclear cells; PBMC+PHA – PBMC treated with phytohaemagglutinin; HeLa – human cervix carcinoma cell line; Fem-x – human melanoma cell line; LS-174 – human colon cancer cell line.

Discussion

Investigation of the antiproliferative action of selected ITCs reported was performed in the presence of human sera (10%), including pooled AB positive sera from healthy donors used in experiments with malignant cells in culture and autologous sera for investigation of antiproliferative effects of ITCs on PBMC. As far as authors are aware the antiproliferative screening of ITCs in the presence of human sera was not performed previously. However, it has been shown that the presence of human umbilical blood sera has stimulatory influence on growth and proliferative capacity of human mesenchymal stem cells, without influence on their morphological and functional characteristics [27]. It has been shown also that both normal and neoplastic cells of nervous origin, cultured in the presence of human sera, have distinct adhesive characteristics, proliferation capacity and antigen expression compared to the same type of cells grown in the presence of calf serum [28]. Accordingly, the proposed experimental design that includes the presence of human sera with putative influence on phenotypic and functional characteristic of biological models could be considered as better approximation of *in vivo* conditions. However, obtained results are in accordance with the previously published data on the antiproliferative action of SFN, BITC and PEITC in HeLa, [29–31] as well as in LS 174 cells [32].

Additionally, cell models used for the selectivity assessment usually include normal cells of the same origin in addition to malignant cells [26] or PBMCs, as proposed in our work. Prioritisation of PBMCs as a model was rationalised by important role of PBMC subpopulations in the immunological control of tumor growth [33] and a possibility to include mitogen stimulated cells as a model of normal cells in the highly proliferative state.

PBMC from healthy individuals, stimulated for proliferation with PHA were similarly sensitive to the cytotoxic action of SFN as low sensitive HeLa and LS 174 cells. This is in consent with published data which show that after 72 h of incubation with 30 mmol m^{-3} of SFN only 16% of T-lymphocytes stimulated with PHA were viable compared to the control [34]. It is also in accordance with the recently published data that the action of BITC is particularly expressed on highly proliferative cells [35].

The obtained results showing lower IC_{50} values for BITC and SFN on PBMC treated with mitogen compared to untreated PBMC are in line with previously published data [14]. Action of PEITC characterised with lower IC_{50} values obtained in PBMC than in PBMC treated with mitogen, suggests different mechanism that needs to be investigated further with putative effects on both malignant and non-malignant lymphoproliferative diseases. Lower IC_{50} values on PBMC compared to cancer cells was most pronounced for BITC as observed also based on the selectivity indexes obtained that were in a wide range between 3.19 for LS 174 cells and 16.57 for Fem-x cells. This is a new result which marks BITC as promising cancer suppressive ITC with the highest selectivity index among the investigated compounds.

The observed differences in the antiproliferative potential of the investigated ITCs could be at least in part due to the differences in kinetics of cellular accumulation. Substantial accumulation within a cell is a general characteristics of the most of the investigated ITCs. However, the kinetics and the level of accumulation strongly depend on the nature of particular ITCs, incubation temperature and glutathione levels in the cell [36]. It was shown on Hepa 1clc7 liver cells that BITC accumulation is a fast process with maximum concentrations reached 30 minutes after the exposure, followed by rapid decline in intracellular concentration leading to complete clearance after 24 h [36]. Contrary to BITC, very slow intracellular accumulation was observed in cells exposed to the same concentrations of SFN with maximum levels reached 12 h after the exposure, followed by the slow decline with half of maximum levels detected at 24 h. This was confirmed in other cell lines treated with the same concentrations of bi-

oactives during 30 minutes. Surprisingly, intracellular levels of BITC were up to 3 times higher compared to the levels of SFN [36]. The capacity of ITCs to induce antioxidant enzymes activity has shown the inverse correlation with the accumulation kinetics and ITCs levels observed 24 h after the exposure, with SFN highlighted as the best inducer of their activity. Contrary to the influence on antioxidant enzymes activity, kinetics of the intracellular transfer was shown to be in direct correlation with the antiproliferative capacities of different ITCs. In a panel of malignant cells of different origin (HL60S, 8662/S, MCF-7, HepG2, HT-29, HaCaT) IC_{50} values obtained after 72 h of continuous exposure to BITC and PEITC were similar to the IC_{50} values obtained after the same period, i.e. 72 h that combines 3 h of direct exposure to ITCs followed by washings and subsequent 69 h long incubation without ITCs [36]. The results obtained for SFN were different in IC_{50} values after 72 h of exposure to SFN 10 times lower compared to the 3h-long exposure followed by 69 h of incubation without ITCs [36]. However, after short exposure to the same concentrations of ITCs, BITC accumulation in cells, levels of reactive oxygen species and antiproliferative capacity are much higher than in SFN treated cells [37]. The observed differences, mostly due to different side chains influencing their lipophilicity have major effects on intracellular action of ITCs. Within a cell all ITCs react directly *via* carbon atom of the –N=C=S group with the cysteine sulfhydryl groups of glutathione (GSH) and proteins. The side chains generally play secondary role, mainly by influencing the electrophilicity of the –N=C=S group and steric effects [38]. With greater ITCs influx and subsequent GSH depletion, cells are more sensitive to the effects of intracellular reactive oxygen species (ROS). On the contrary, slower influx and consequent slow decrease on GSH level could act as a signal for glutathione-S-transferase activation, resulting in higher GSH levels [38]. Malignant phenotype is characterised by high levels of oxidative stress, mainly due to high levels of ROS and disturbed ratio between reduced and oxidised form of GSH [39]. The persistent oxidative stress in cancer cells sensitizes them to stress or apoptotic effects of anticancer drugs, which often generate ROS, because they are already near a threshold for tolerating ROS. It is not the case with normal cells, with lower production of H_2O_2 [40]. It seems that the fate of cancer cells to survive the effects of ITC depends on constitutive levels of ROS and/or glutathione, the type, dose and accumulation kinetics of ITC the cells were exposed to, resulting in the difference in sensitivity of different cells types and the difference in ITCs selectivity regarding their antiproliferative action.

It has been shown previously that chemopreventive action of SFN, as the strongest inhibitor of phase I enzymes and inductor of phase II enzymes in this group of bioactives, is mediated mainly by the modulation of carcinogen metabolism [15]. Accordingly, SFN is the major ITC highlighted for the use in primary prevention at low dietary relevant doses, *via* its major dietary sources, including broccoli. However, the selectivity indexes for the action of SFN are lower compared to other investigated ITCs suggesting that SFN at higher doses (as isolated molecule or in enriched extracts) could affect proliferation of immunocompetent cells. It should be noted that the obtained results rationalize further investigation of putative beneficial effects of SFN on the suppression of limphoproliferative or autoimmune diseases.

Compared to SFN and PEITC, BITC has shown better characteristics as chemopreventive agent acting on the proliferation of cancer cells. The pronounced selectivity in antitumor action of this compound, by comparison of its effects on malignant cells and PBMC, favors this particular ITC for the use as cancer suppressive chemopreventive agent. BITC is a major ITC of garden cress, and it is not present in broccoli or in many other dietary sources of glucosinolates and isothiocyanates [24]. Thus, in addition to its beneficial effects the distribution in dietary plants should be taken into account in evidence-based personalised nutrition recommendation and the use of dietary sources of BITC in secondary or tertiary chemoprevention. Other dietary sources of glucotropeolin and BITC that are not part of westernised diet should be promoted to provide higher intake of this bioactive compound in subjects with high cancer risk or cancer patients.

Conclusion

Numerous biological effects of ITCs suggest their active role in dietary prevention of malignant and other chronic diseases that represent major burden to health worldwide. Our data contribute to the rationale for future comprehensive studies that could eventually lead to more specific dietary guidance and recommendations for increased intake of vegetables containing particular glucosinolates and ITCs, targeted to the specific populations such are cancer patients or subjects at high cancer risk. Further research and conclusions based on human intervention trials are needed to provide additional scientific evidence for the beneficial effects of long-term intake of BITC or its dietary sources in cancer patients, as agent in tertiary chemoprevention or even as a complementary therapeutic. ITCs are also considered as a good starting point for synthesis of functional analogues that will enhance their biological activity. However, the critical assessment of their safety should be included in the evaluation of their potential use in primary, secondary and tertiary prevention of cancer, as a part of balanced diet, functional component of functional foods and dietary supplements, chemopreventive agents and therapeutics. Screening of selectivity by applying a model presented in this work is a plausible approach for the preclinical evaluation of safety of both natural ITCs and synthesised analogues of these bioactive compounds.

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Influence of open surgical and endovascular abdominal aortic aneurysm repair on clot quality assessed by ROTEM® test

Uticaj otvorene i endovaskularne rekonstrukcije aneurizme abdominalne aorte na kvalitet koaguluma meren ROTEM® testom

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Abstract

Introduction/Aim. The disturbances in hemostasis are often in open surgical repair (OR) and endovascular repair (EVAR) of an abdominal aortic aneurysm (AAA). These changes may influence the perioperative and early postoperative period inducing serious complications. The aim of this study was to compare the impact of OR and EVAR of AAA on clot quality assessed by rotational thromboelastometry (ROTEM®) tests. **Methods.** The study included 40 patients who underwent elective AAA surgery and were devided into two groups (the OR and the EVAR group – 20 patients in each group). The ROTEM® test was performed in 4 points: point 1 – 10 min before starting anesthesia in both groups; point 2 – 10 min after aortic clapming in the OR group and 10 min after the stent-graft trunk release in the EVAR group; point 3 – 10 min after the releasing of aortic clamp in the OR group and 10 min after stent-graft placement and releasing the femoral clamp in the EVAR group; point 4 – one hour after the procedure in both groups. Three ROTEM® tests were performed as: extrinsically activated assay with tissue factor (EXTEM), intrinsically activated test using kaolin (INTEM), and extrinsically activated test with tissue factor and the platelet inhibitor cytochalasin D (FIBTEM). All tests included the assessment of the maximum clot firmness (MCF) and the platelet component of clot strength was presented as maximal clot elasticity (MCE). **Results.** No significant difference in age, gender and diameter of AAA between groups was found. The time required for the procedure was significantly longer and loss of blood was greater in the OR group than in the EVAR group ($p < 0.001$). The significant deviation of MCF values in EXTEM test was found mainly in the point 3 ($p \leq 0.004$) with significant difference between groups ($p < 0.001$). A significant difference of MCF values in INTEM test between groups was found in the points 3 and 4 ($p < 0.001$), which were dose-dependent by heparin sulfate. The MCF values in FIBTEM test were more prominent in the OR group than in the EVAR group without significant difference. The significant changes of MCF values in the FIBTEM test were found during time in both groups ($p < 0.001$). The values of MCE were lower in both groups, but without significant changes and difference between groups ($p = 0.105$). **Conclusion.** The disorders of hemostatic parameters assessed by ROTEM® tests are present in both the OR and the EVAR groups being more prominent in OR of AAA. Vigilant monitoring of hemostatic parameters evaluated by ROTEM® tests could help in administration of the adequate and target therapy in patients who underwent EVAR or OR of AAA.

Key words:
aortic aneurism, abdominal; vascular surgical procedures; blood coagulation; blood loss, surgical; platelet function tests.

Apstrakt

Uvod/Cilj. Poremećaji hemostaze su česti u otvorenoj (OR) i endovaskularnoj rekonstrukciji (EVAR) aneurizme abdominalne aorte (AAA). Ove promene mogu uticati na perioperativni i rani postoperativni period uzrokujući ozbiljne komplikacije. Cilj studije bio je da se uporedi uticaj OR i EVAR AAA na kvalitet koaguluma procenjen testovima rotacione tromboelastometrije (ROTEM®). **Metode.** Studija je obuhvatila 40 bolesnika kod kojih je izvedena elektivna operacija AAA, i koji su bili podeljeni u dve grupe (OR i EVAR grupa – po 20 bolesnika u svakoj grupi). ROTEM® test urađen je u 4 tačke: tačka 1 – 10 min pre uvođenja u anesteziju u obe grupe; tačka 2 – 10 min nakon klemovanja aorte u OR grupi i 10 min nakon otpuštanja trunkusa stent grafta u EVAR grupi; tačka 3 – 10 min nakon otpuštanja kleme sa aorte u OR grupi i 10 min nakon plasiranja stent grafta i otpuštanja klema sa femoralnih arterija u EVAR grupi; tačka 4 – jedan sat nakon operacije u obe grupe. Izvedena su tri ROTEM® testa: spoljašnji aktivacioni esej sa tkivnim faktorom (EXTEM), unutrašnji aktivacioni test

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sa kaolinom (INTEM) i spoljašnji aktivacioni test sa tkivnim faktorom i trombocitnim inhibitorom citohalazinom D (FIBTEM). Svi testovi uključili su procenu maksimalne čvrstine koaguluma (MČK) i trombocitna komponenta jačine koaguluma predstavljena je maksimalnom elastičnošću koaguluma (MEK). **Rezultati.** Nije bilo značajne razlike između grupa u godinama starosti i polu bolesnika i dijametru AAA. Trajanje operacije bilo je značajno duže kao i količina izgubljene krvi u OR nego u EVAR grupi, ($p < 0{,}001$). Nađene su značajne promene u MČF vrednostima u EXTEM-u, naročito u tački 3 ($p \leq 0{,}004$), sa značajnom razlikom između grupa ($p < 0.001$). Nađena je značajna razlika između grupa u vrednostima MČK u INTEM-u u tačkama 3 i 4, što je bilo dozno zavisno od heparina. Vrednosti MČK u FIBTEM-u bile su izraženije u OR grupi nego u EVAR grupi, ali bez značajne razlike između grupa. Značajne promene u MČK vrednostima u FIBTEM-u tokom vremena nađene su u obe grupe ($p < 0{,}001$). Vrednosti MČK bile su niže u obe grupe, ali bez značajnih promena i bez značajne razlike između grupa ($p = 0{,}105$). **Zaključak.** Poremećaji parametara hemostaze procenjenih ROTEM® testovi bili su prisutni u OR i EVAR proceduri, ali su bili izraženiji u OR AAA. Vigilno praćenje parametara hemostaze uz pomoć ROTEM® testova može pomoći u primeni adekvatne i ciljne terapije kod bolesnika podvrgnutih EVAR i OR AAA.

Ključne reči:
aorta, abdominalna, aneurizma; hirurgija, vaskularna, procedure; krv, koagulacija; krv, hirurški gubitak; trombociti, funkcijski testovi.

Introduction

The abdominal aortic aneurysm (AAA) is a common disease in the era of a modern medicine and surgery also and ussualy occurs in patients with different comorbidity states. Those during AAA repair as well as perioperative hemostatic disturbances are common and could deterioate during surgery and in the early postoperative period. In the perioperative period, disturbances of hemostatic parameters such as elevated plasma levels of factor VIII, fibrinogen, disorders of fibrinolysis with elevated level of d-dimer and platelet hyperactivity are reported 1, 2.

The elective open surgical repair (OR) of infrarenal AAA is associated with a perioperative mortality rate of 3% to 10% 3. Perioperative bleeding, general hypothermia, the use of anesthetics, as well as ischemia reperfusion injury result in a systemic inflammatory response with microvascular and macrovascular thrombosis that may cause myocardial infarction, stroke, thromboembolism, and multiple organ failure. Thereby, all of these could increase the operative mortality rate 4, 5.

As compared to OR, endovascular aneurysm repair (EVAR) of an AAA represents a safer alternative in anatomically suitable patients. Thus, the EVAR-1, EVAR-2, and Dutch Randomized Endovascular Aneurysm Management trials reported a 60% reduction in perioperative mortality rate in EVAR compared to OR of AAA, but without difference in a long term mortality rate postoperatively 6, 7.

A systematic inflammatory response is observed in a significant number of patients after EVAR. The intensity of inflammation, assessed mainly by the postoperative high level of C-reactive protein (CRP) values, correlates with the presence of a cardiovascular or any other adverse event during the first 30 days after the procedure 8.

The inflammatory response in EVAR could lead to hemostatic disturbances, similar to those seen after OR of AAA, although it is less invasive surgery 9, 10. The explanation for this lies in the use of radiological contrast medium, intra-arterial implants (stent grafts) 11, 12, as well as manipulations with radiological devices such as introducers and cathethers for intra-arterial – endovascular procedures 9, 13. The existence of specific complications in EVAR – endoleak, could also lead to hemostatic disturbances and coagulopathy 14.

The aim of this study was to compare the impact of OR of AAA and EVAR on hemostasis, assessed by quality of blood clot measured by rotational thromboelastometry – ROTEM® (ROTEM® delta, TEM® International GmbH, Munich, Germany) parameters.

Methods

This prospective observational study included 40 patients who underwent elective AAA surgery, and divided into two groups. The first group included 20 patients with OR of AAA (OR group) and the second one included another 20 patients with EVAR of AAA (EVAR group). Written informed consent was obtained from all patients after a detailed description of the procedure.

Inclusion criteria were as follows: asimptomatic AAA of 5.5 cm in diameter and more, confirmed by multi-slice computed tomography (MSCT) scan which was also a parameter for the technical feasibility of performing EVAR procedure. The study excluded patients with any known significant disorder in coagulation, patients on oral antocoagulant and antiplatelet therapy in the last 7 days, patients with chronic renal failure (serum creatinin higher than 200 μmol/L), liver disease, acute and/or active inflammatory states and malignancy. The classification of the American Society of Anesthesiologists (ASA) was used for the assessment of perioperative risk.

The procedure was performed under balanced general anesthesia (propofol, isofluran) in all patients. All patients received a dose of 100 IU/kg of heparin sulfate 15 min before aortic clamping in the OR group and 5000 IU in a single dose 15 min before releasing of stent-graft trunk in the EVAR group (point 2 in both groups). Patients in the OR group received protamine dose of 1 mg/kg for the neutralization of heparin sulfate after the end of aortic clamping (10 min before the point 3). In the EVAR group point 3 was defined as finished endograft placement. Perioperative monitoring was performed in both group. During the operative procedure each patient received crystalloids substitution of 15 mL/kg/h. Blood derivatives and blood substitution therapy were administered according to the results of hemoglobin level perioperatively and amount of intraoperative bleeding.

EVAR procedure: after surgically exposing both femoral arteries for EVAR the angiography was performed in all patients. The endoprosthesis was inserted and released into the aorta using extra stiff 300 cm long 0.035 inch guidewire (Lunderquist Extra Stiff Guide Wire, Cook medical, USA), controlled by angiography. The exclusion of the aneurysm sac was confirmed by angiography immediately following the procedure. Primary success criteria were intraoperative survival rate, the absence of an open surgical conversion, the exclusion of aneurysmal sac, the absence of type I or III endoleaks. We used one or more GORE® EXCLUDER® AAA Endoprosthesis (W. L. Gore & Associates, Inc. Arizona, USA) for all patients. A bifurcated prosthesis was implanted in all patients.

OR technique: through medial laparotomy and transperitoneal approach the AAA and iliac blood vessels were exposed in all patients. The femoral blood vessels were exposed through inguinal approach on the both sides if the aortobifemoral reconstruction was necessary. After heparin sulfate administration a proximal and distal aortic clamping was performed. After aortotomy the aortic reconstruction was done in a typical way. Proximal anastomosis was created endoaneurysmatically end-to-end sided with tubular graft. In cases with aneurysm in iliac artery (one or both), or in cases with occlusive iliac disease a distal anastomosis was created end-to-end or end-to-side between bifurcated graft and iliac/femoral arteries. After finished reconstruction, all patients without extensive bleeding received protamine. The aneurysmatic sac was sutured over the graft.

The ROTEM® tests were performed several times in all patients: before intervention, perioperatively and postoperatively.

Three ROTEM® tests were performed: extrinsically activated assay with tissue factor (EXTEM), intrinsically activated test using kaolin (INTEM), and extrinsically activated test with tissue factor and the platelet inhibitor cytochalasin D (FIBTEM) (Figure 1). Reference ranges for the tests' parameters have been previously determined in a multi-centre investigation 15. The ROTEM® test was performed: 10 min before starting anesthesia in both groups – point 1; 10 min after aortic clamping in the OR group and 10 min after of stent-graft trunk release in the EVAR group – point 2; 10 min after releasing the aortic clamp in OR group and 10 min after finished stent-graft placement and releasing the femoral clamp in the EVAR group – point 3; and one hour after the operation in both groups – point 4.

Blood samples for ROTEM® analysis were collected in a standard coagulation tube syringe containing a 0.106 M citrate solution, with blood/citrate ratio of 9 : 1.

ROTEM® tests were performed according to the manufacturer's recommendations, and the analyses were performed within 5 min of blood sampling. The EXTEM, INTEM and FIBTEM analyses included the assessment of the maximum clot firmness (MCF). The platelet component of clot strength was calculated as follows: MCE (platelet) = MCE (EXTEM) – MCE (FIBTEM). Maximum clot elasticity (MCE) was calculated by formula (MCFx100)/(100-MCF).

All statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL). The data were presented in number (%), median (range) and mean ± standard deviation. The comparison between groups including demographic and clinical characteristic of the patients was assessed with $\chi 2$ test ant t-test. Multiple comparisons between measured variables in different points were assessed with Bonfferoni test method. The comparison between each variable was performed using logistic regression and ANOVA test. P value less than 0.05 was considered as significant.

Results

The demographic and clinical characteristics of patients are presented in Table 1.

There was no in-hospital mortality in both groups of patients. No significant difference in age, gender and diameter of

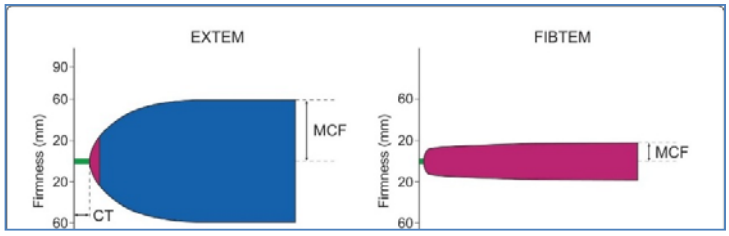

Fig. 1 – The ROTEM® analyses.

EXTEM® test (extrinsically activated test) and FIBTEM® test (fibrin clot obtained by platelet inhibition with cytochalasin D); CT – clot time. The maximum clot firmness (MCF, mm) represents the total amplitude of the clot.

Table 1

The demographic and clinical characteristics of operated on patients

Patients	OR (n = 20)	EVAR (n = 20)	*p*
Age (years), $\bar{x}$ ± SD	66.2 ± 7.4	68.8 ± 11.01	0.253
Sex M/F, n (%)	27/3 (11.1%)	29/1 (3.48%)	0.630
Aneurysm diameter (cm), $\bar{x}$ ± SD	6.6 ± 2.02	7.03 ± 1.23	0.093
Duration of operation (min), $\bar{x}$ ± SD	167.0 ± 58.1	102.9 ± 34.8	< 0.001
Bleeding volume (mL), $\bar{x}$ ± SD	1058.21 ± 722.9	389.6 ± 161.6	< 0.001
ASA class III or IV, (%)	56.5	93.1	< 0.001

Abbreviation: OR – open surgical repair; EVAR – endovascular aortic repair; AAA – aortic abdominal aneurysm; ASA - American Society of Anesthesiologists.

AAA between groups was found. The time required for the procedure was significantly longer in the OR group than in the EVAR group. Blood loss during the procedure was present in both groups, and significant difference between groups was found. As it was expected, blood loss during surgery was greater in the OR group ($p < 0.001$). Only one patient in the EVAR group required blood transfusion of erythrocyte concentrate. There were more patients with ASA class III or IV in OR group with significant difference between groups ($p < 0.001$).

The values of MCF in EXTEM test in each point of measurement are presented in Table 2 and analysis of values comparison is presented in Table 3. The dynamic of MCF values change in EXTEM test in both groups and in all points of assessment (dynamics in clot quality in each point of measurement) is shown in Table 2. There were no significant deviations in MCF values in EXTEM in the EVAR group and these values were in the normal ranges mainly. Significant changes were in MCF value in EXTEM test in the OR group during procedure with maximum noted deviation in the point 3 ($p \leq 0.004$) (Table 3), with significant difference between groups ($p < 0.001$). The values of MCF in EXTEM test in the OR group after the point 3 gradually returned in the normal ranges in the point 4 (one hour after procedure).

The values of MCF in INTEM test in each point of measurement are presented in Table 4 and analysis of values comparison is presented in Table 5. The dynamic of MCF

Table 2

The values of MCF in EXTEM test in each time point of measurement in both groups

Group	Time point	Mean	SD	Median	Range
EVAR (n = 20)	1	66.93	5.982	67.0	56–79
	2	63.81	9.108	65.5	35–76
	3	58.50	16.403	61.5	7–74
	4	58.71	17.709	63.0	7–78
OR (n = 20)	1	64.54	10.819	67.0	12–76
	2	62.56	7.048	61.0	48–78
	3	41.77	12.313	43.0	6–59
	4	53.77	9.201	54.0	18–73

OR – open surgical repair; EVAR – endovascular aortic repair; EXTEM – extrinsilally activated assay with tissue factor; MCF – maximum clot firmness (normal range 49–71 mm).
Time points: 1 – 10 min before starting anesthesia in both group; 2 – 10 min after aortic clamping in the OR group and 10 min after of stent-graft trunk release in the EVAR group; 3 – 10 min after releasing the aortic clamp in OR group and 10 min after finished stent-graft placement and releasing the femoral clamp in the EVAR group; 4 – one hour after the operation in both groups.

Table 3

Bonferroni test for multiple comparison (MCF values in EXTEM)

Group	Time point	Time point			
		1	2	3	4
		Sig.	Sig.	Sig.	Sig.
EVAR	1		1.000	0.691	0.397
	2	1.000		1.000	0.647
	3	0.691	1.000		1.000
	4	0.397	0.647	1.000	
OR	1		1.000	0.000	0.116
	2	1.000		0.000	0.061
	3	0.000	0.000		0.004
	4	0.116	0.061	0.004	

Based on estimated marginal means; Dependent variable: tExtem MCF; Adjustment for multiple comparisons: Bonferroni statistical significance;
For abbreviations and explanations see Table 2.

Table 4

The values of INTEM MCF in each time point of measurement in both groups

Group	Time point	Mean	SD	Median	Range
EVAR (n = 20)	1	66.25	5.183	65.50	56–78
	2	62.31	9.112	64.50	42–80
	3	56.81	8.998	59.50	40–67
	4	51.88	15.064	52.00	15–72
OR (n = 20)	1	64.87	6.400	66.00	47–76
	2	41.05	20.109	45.00	6–72
	3	55.63	11.504	57.50	8–70
	4	62.49	6.104	63.00	47–73

INTEM – intrinsically activated test using kaolin; MCF – maximum clot firmness (normal range 52–72 mm).
For abbrevations and explanations see Table 2.

values change in INTEM test in both groups and in all points of assessment (dynamics in clot quality in each point of measurement) is shown in Table 4.

Analysis of MCF values in INTEM test showed different dynamics of clot quality due to influence of heparin sulfate on hemostasis and coagulation status as well. After administration of heparin sulfate (point 2) in the EVAR group there was an evident slow decrease in MCF values which continued to decline in the point 3 and 4 (patients in the EVAR group did not received protamine for the neutralization of heparin sulfate). After a decrease in MCF values in the point 2 in the OR group, a normalisation of MCF values in the point 3 and 4 was evident, which were in normal ranges in the point 4 (protamine administration).

Analysis of MCF values in FIBTEM test showed impact of fibrinogen on the clot firmness during OR and EVAR of AAA. It was evident that the value of MCF in FIBTEM test declined slowly in EVAR group with normalisation after one hour postoperatively (point 4). However, normal ranges were in each point. The changes in MCF values in FIBTEM test were more prominent in the OR group, mainly in the point 3. The dynamics of MCF values in FIBTEM test in both groups and in all points of assessment (dynamics in clot quality in each point of measurement) are shown is Table 6.

A significant difference in MCF values changes in FIBTEM test in all patients was found ($F = 50.402$; $p < 0.001$), with no significant difference between groups ($F = 0.179$; $p = 0.674$). However, there was a significant impact of operative procedures (OR and EVAR) on MCF values changes in FIBTEM test in time ($F = 4.986$; $p < 0.001$).

Using the above mentioned formula in Methods, the platelet component of clot strength and MCE were calculated. Tables 7 and 8 represent the dynamics of clot elasticity and the platelet component of clot with its influence on clot quality in each point of measurement in both groups.

The significant influence of operative procedures on MCE value changes during the time was not found ($F = 1.853$; $p = 0.105$). The amount of platelet and other factors in the clot and their influence on clot elasticity was without significant changes in both groups, and with no difference between groups ($p = 0.105$).

Table 5

Bonferroni test for multiple comparison (MCF values in INTEM)

Group	Time point	Time point			
		1	2	3	4
		Sig.	Sig.	Sig.	Sig.
EVAR	1		1.000	0.013	0.000
	2	1.000		1.000	0.183
	3	0.013	1.000		0.840
	4	0.000	0.183	0.840	
OR	1		0.000	0.000	0.782
	2	0.000		0.000	0.000
	3	0.000	0.000		0.012
	4	0.782	0.000	0.012	

Based on estimated marginal means; Dependent variable: Intem MCF; Adjustment for multiple comparisons: Bonferroni.
For abbrevation see and explantations Tables 2 and 4.

Table 6

The dinamycs of MCF values (mm) in FIBTEM test in both groups

Group	Time point			
	1	2	3	4
OR	20.53	16.53	13	17.89
EVAR	21.89	18.5	18.31	18.37

FIBTEM – fibrin clot obtained by platelet inhibition with cytochalasin D; MCF – maximum clot firmness (normal range 9–25 mm).
For abbrevations and explanations see Table 2.

Table 7

Maximum clot elesticity (MCE) in each time point of measarement in the EVAR group

MCE	Time point			
	1	2	3	4
Extem	202.38	177.23	140.96	142.18
Fibtem	27.89	22.69	22.41	22.5
Platelet	174.49	153.54	118.55	119.68

For abbrevation and explantations see Tables 2 and 6.

Table 8

Maximum clot elasticity (MCE) in each time point of measurement in OR group

MCE	Time point			
	1	2	3	4
Extem	198.86	167.09	71.73	116.3
Fibtem	25.83	19.66	14.94	21.78
Platelet	173.03	147.43	56.84	94.52

For abbrevation and explantations see Tables 2 and 7.

Discussion

AAA is almost always associated with disorders of hemostasis. It was reported that a lot of patients with AAA had elevated serum levels of d-dimer, fibrinogen, as well as altered platelet functions. Also, a relationship between diameter of AAA, thrombus volume and serum level of d-dimer was found [16]. Aging may affect the changes in hemostasis also [17]. All those disorders can lead to disturbances in hemostasis during the surgical procedure and/or after the procedure with dangerous consequences (bleeding, thromboembolism, etc). Generally, OR of AAA is accompanied with greater trauma of the body as compared to EVAR. Also, the implantation of prosthetic material in both OR and EVAR techniques has an impact on hemostasis. General hypothermia during surgical procedure has influence on hemostasis and can lead to hemostatic disturbances and coagulopathy [18]. Bleeding and blood substitution therapy lead to changes in hemostatic parameters *per se* [19]. The immune system is affected also with altered immunologic parameters. A high concentration of IL-1, IL-6 and TNF in AAA, and elevated serum level of CRP were shown [20, 21].

In comparison to OR of AAA, EVAR technique is less traumatic with lower incidence of serious perioperative and early postoperative complications [22]. However, the usage of radiographic contrast medium could change the homeostasis of hemostasis and platelet functioning [23]. Manipulation into arterial blood vessels and endoprothesis implantation lead to the activation of immune system as well as disturbances in hemostasis with development of well known post-implantation syndrome [23, 24]. In contrast to OR of AAA whereas the thrombus is removing with all active substances, in EVAR technique it remains *in situ* and represents a source of inflammatory mediators with all their systemic effects. The existence of specific complications in EVAR (endoleaks), especially the endoleak type 2, could lead to a leakage of cytokines into systemic circulations. This is one of the reasons for the hemostasis disturbances occurrence, and in some cases the appearance of coagulopathy [25]. All mentioned suggests that the changes in hemostasis during and after EVAR are similar or slightly less than those during OR of AAA.

The results of our study showed the active changes in the coagulation system throughout both EVAR and OR of AAA. We analyzed the firmness of clot in several points in EVAR and OR of AAA assessed with ROTEM® tests. This is the first study which assessed the clot firmness in all EXTEM, INTEM and FIBTEM tests in both EVAR and OR of AAA. MCF values by ROTEM® were evaluated and reported in the study with cardiac surgery patients but not in all tests [26, 27]. These studies suggested that ROTEM® may be used to manage anticoagulation and transfusion therapy for bleeding. The tests we used for MCF assessment were EXTEM, INTEM and FIBTEM, and we calculated the MCE with mathematic model, as well as compound of platelet in clot elasticity – MCE platelet. Generally, we found that both techniques, EVAR and OR, for AAA solving changed the whole system of hemostasis, evaluated by ROTEM® tests. Also, we found a change in the blood clot composition in term of clot quality reduction. The reduction in clot quality (MCF) was found in all tests in both groups. It was reported an active fibrinolysis, platelet and coagulation factors consumption in patients with aortic pathologies who were treated by EVAR technique [28]. We found a coagulation disturbances not only in the EVAR but in the OR group, too.

The equivalent of MCF value in ROTEM® tests is a value of maximum amplitude (MA) in thrombelastography (TEG®) test. The changes in these parameters in patients who underwent AAA reconstruction were reported by Franks et al. [29]. They found a reduced clot quality during OR of AAA, whereas clot quality has been improving two hours after EVAR technique. Our findings are similar but not identical. In the OR groups Franks et al. [29] showed continuous reduction of clot quality in EXTEM test during surgical procedure and two hours after. We found a similar trend of MCF, however clot quality had started improving one hour after the procedure with subsequent upward trend. This could be explained by active substitution of blood and blood derivates in our patients during and after the surgery. The intraoperative changes in MCF values in EXTEM test were more prominent in the OR group than in the EVAR group, whereas clot quality and elasticity were decreasing until the end of the procedure. If the blood loss is greater during surgery, the clot quality and elasticity will be more changed. That was the conlusion of Plotkin et al. [30] also, who sugessted that clot quality could be possible predictor of bleeding and blood transfusion. Our results correspond to these findings. In the Medline database we could not find a study that analyzed MCF parameters in INTEM test during EVAR nor during OR of AAA. However, Mittermayr et al. [31] assessed influence of heparin sulfate on INTEM parameters (ROTEM®) and reported dose dependence of parameters. In our study we found significant difference in MCF values in INTEM test in both groups and between groups. Also, MCF values in INTEM test were influenced by heparin sulfate. Both groups received heparin sulfate in the point 2 when the MCF values dropped. The declining in MCF values in the EVAR group was linear until the end of procedure. After finished EVAR, MCF values did not normalized due to the absence of heparin sulfate neutralization by protamine. These findings were similar with dynamics of clot time (CT) values in INTEM test reported by Mittermayr et al. [31]. In the OR group the values of MCF started normalizing after the point 3 (administration of protamine) and were in normal ranges one hour after the end of the procedure. Parameters in INTEM test were dose dependent by heparin sulfate, so INTEM test can be used for monitoring of heparin sulfate and protamine administration.

The influence of fibrinogen on coagulation could be assessed by FIBTEM parameters, as well as concentration and amount of fibrinogen and platelet in clot, and clot elasticity and quality. Besides analyses of the clot quality and elasticity it is important to determine the influence of other coagulation factors on coagulation. The assessment of ethiopathogenesis of the quality changes in hemostasis should includes factor XIII [32], usage of antiplatelet drugs, fluid resuscitation [33], body temperature during surgery, duration of the procedure, bleeding, blood substitution therapy, radiological contrast medium in EVAR [19, 22], etc, in addition to ROTEM® and TEG®

analyses. Schochl et al. [34] reported significantly decreased values of MCF in FIBTEM test (less than 6 mm) in patients with trauma and massive bleeding. These values of MCF should correspond values of fibrinogen less than 1 g/L. We found that MCF values in FIBTEM test were more prominent in the OR group than in the EVAR group without significant difference between groups. However, there were significant changes of MCF values in FIBTEM test during the time in both groups of patients ($p < 0.001$), and significant impact of group on MCF values in FIBTEM test ($p < 0.001$). The greatest decline in MCF values in FIBTEM test was noted in the point 3, mainly in the OR group, when the largest amount of bleeding was recorded.

Shenkman et al. [35] recorded that the clot quality may be improved by administration of factor XIII in cases with low level of platelet, but improving of clot quality was limited. In such cases where the level of platelets was extremely low, clot quality could not be improved. Our results showed that clot quality declined during intervention, and that was more significant in the OR than in the EVAR group due to higher blood loss in the OR group of patients. The amount of platelet and other factors in the clot and their influence on clot elasticity were without significant changes in both groups without difference between groups. The ROTEM® test during EVAR and OR can indicate a disturbances in hemostasis and may predict possible conversion of EVAR in OR of AAA. Vigilant and timely monitoring of hemostatic parameters with the adequate and target substitution of blood and blood products therapy in patients who underwent EVAR and OR of AAA can reduce disturbances in hemostasis and subsequent lower incidence of complications.

Conclusion

The results of our study show active changes in the coagulation parameters during both EVAR and OR of AAA evaluated by ROTEM® analysis. In the OR group a significant deviation of MCF values was found with maximum deviation noted in the point 3. The reduction in clot quality (MCF) was present in all tests in both groups. A significant difference of MCF values in INTEM test between the EVAR and the OR group was found. The MCF values in INTEM test were dose dependent by heparin sulfate, so INTEM test can be used for monitoring of heparin sulfate and protamine administration. The MCF values in FIBTEM test were more prominent in the OR group than in the EVAR group but without significant difference. The significant changes of MCF values in FIBTEM test during time were found in both groups. A decline in MCF values in FIBTEM test was noted in the OR group patients when the largest amount of bleeding was recorded. The clot quality declined during intervention in both groups, mainly in the OR group due to higher blood loss. The values of MCE were lower in both groups, but without significant changes and without difference between the groups. Vigilant and timely monitoring of hemostatic parameters by ROTEM® tests could help in administration of the adequate therapy with subsequent reduction of disturbances in hemostasis and incidence of complications.

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Sodium intake and dietary sources of sodium in a sample of undergraduate students from Novi Sad, Serbia

Unos natrijuma i nutritivni izvori natrijuma u uzorku studenata iz Novog Sada, Srbija

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Abstract

Background/Aim. Data on sodium intake and sources of sodium in the diet in Serbia are limited. The aim of this study was to estimate the sodium intake and identify the sources of sodium in the diet of undergraduate students attending the University of Novi Sad. **Methods.** Students completed a questionnaire to gather data on their gender, age and university faculty attended, and then a 24 h dietary recall. The sodium intake of the students was calculated using the dietary recall data and data on the sodium content of foods. The contribution of different food groups as well as of specific foodstuffs to the total sodium intake was calculated. **Results.** The mean estimated sodium intake of the students was 3,938.5 ± 1,708.1 mg/day. The sodium intake of 89.1% of the surveyed students exceeded the guideline for sodium intake, the majority of the sodium coming from processed foods (78.9% of the total sodium intake). The food groups that contributed the most to the total sodium intake of the students were meat and meat products (21.7%) and cereals and cereal-based products (18.6%). Bread and other bakery products were responsible for 13.1% of the total sodium intake. **Conclusion.** High sodium intake in students of the University of Novi Sad puts them at high risk of developing high blood pressure. The food industry should work towards reformulating products with high sodium content, especially bread and other bakery products. Efforts should be taken to reduce sodium intake among undergraduate students in Novi Sad.

Key words:
sodium, dietary; risk factors; students; serbia; food habits.

Apstrakt

Uvod/Cilj. Podaci o unosu natrijuma i izvorima natrijuma u ishrani stanovništva Srbije su ograničeni. Cilj ovog istraživanja bio je da se proceni unos natrijuma i da se identifikuju izvori natrijuma zastupljeni u ishrani studenata Univerziteta u Novom Sadu. **Metode.** Studenti su popunili upitnik kojim su prikupljeni opšti podaci (pol, uzrast, pohađani fakultet), a potom i anketu ishrane po sećanju za 24 časa. Unos natrijuma izračunat je na osnovu podataka iz ankete ishrane i podataka o sadržaju natrijuma u konzumiranim namirnicama. Izračunat je i doprinos različitih grupa namirnica, kao i doprinos pojedinih namirnica ukupnom unosu natrijuma. **Rezultati.** Prosečni procenjeni unos natrijuma među studentima bio je 3 938,5 ± 1 708,1 mg/danu. Od ukupnog broja, 89,1% ispitanika unosilo je više natrijuma od preporučene količine. Natrijum u ishrani studenata u najvećem procentu poticao je iz industrijski prerađenih namirnica (78,9% ukupnog unosa natrijuma). Grupe namirnica koje najviše doprinose ukupnom unosu natrijuma među studentima bile su meso i proizvodi od mesa (21,7%) i žitarice i proizvodi od žitarica (18,6%). Hleb i drugi pekarski proizvodi bili su odgovorni za 13,1% ukupnog unosa natrijuma. **Zaključak.** Visok unos natrijuma među studentima Univerziteta u Novom Sadu predstavlja faktor rizika od razvoja hipertenzije. Industrijski prerađene namirnice sa visokim sadržajem natrijuma, poput hleba i drugih pekarskih proizvoda, trebalo bi reformulisati. Potrebno je sprovesti obrazovne i promotivne aktivnosti kako bi se unos natrijuma u studentskoj populaciji Univerziteta u Novom Sadu smanjio.

Ključne reči:
natrijum, unos hranom; faktori rizika; studenti; srbija; ishrana, navike.

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Introduction

Hypertension is the leading cause of death worldwide, responsible for 13% of global deaths and close to 4% of disability-adjusted life years [1]. Globally, 40% of adults aged over 25 suffered from high blood pressure in 2008 [2].

High sodium intake causes blood pressure elevation and is a proven modifiable risk factor for hypertension [3]. The World Health Organization (WHO) considers the reduction of sodium intake to be one of the ten "best buy" (cheap, feasible and cost-effective) interventions in reversing the global epidemic of noncommunicable diseases [2]. The current WHO guideline for sodium intake is 2,000 mg/day (5 g of salt *per* day) [4], but the mean sodium intake varies by country and is almost always above the guideline amount [5,6]. Most of the sodium in the "Western diet" comes from processed foods (75%), while only around 10% comes from sodium added to food during cooking or at the table [6–8].

Serbia, as many developing countries, is currently facing an ongoing nutrition transition and a growing burden of noncommunicable diseases. The estimated prevalence of hypertension among adults in Serbia is 46.5% [9]. In the Autonomous Province of Vojvodina, the northernmost part of Serbia, the prevalence of hypertension among adults aged 45 and older is estimated to be 65.5% and in Novi Sad, the regional capital, the prevalence is as high as 70% [10]. Data on sodium intake and sources of sodium in the diet are limited, labeling of salt content in retail food is not required by national legislation and, to date, no activities have been undertaken to raise awareness or reduce sodium intake on the national level.

The aim of this study was to estimate the sodium intake and identify the sources of sodium in the diet of undergraduate students from Novi Sad.

Methods

For this cross-sectional study data were collected during the 2011/12 academic year. Students attending the University of Novi Sad were eligible to participate in the study and there were no exclusion criteria. The students were informed that their participation in the study was voluntary. The response rate was 94% (17 of the students refused to participate in the study).

The Ethical Committee of the University of Novi Sad ruled that specific approval or signed consent from the participants was not required. The management of the University of Novi Sad gave permission for the study to be conducted.

The students completed a questionnaire to give data on their gender, age and faculty attended, and then on a 24-hour dietary recall (24 h DR) in written form. The students were asked by a trained interviewer to write down all the foods and beverages they had consumed during the previous day (24 h), as well as the amounts where possible. After that, the interviewer prompted the students to remember the foods and beverages usually not reported during the 24 h DR (beverages, sweets, salty snacks, fruits, vegetables, bread and cheese) [11]. Where applicable, the amounts of foods and beverages consumed were estimated using a portion size photo album [12] shown to the participants by the interviewer.

The data on the sodium content of foods and beverages consumed by the participants were obtained from the national food composition database [13] and from the regional food composition databases [14, 15], in cases where specific national data were not available.

An application specially designed for the purpose of this study was used to calculate sodium intake using the data on the sodium content of foods and beverages from the food composition databases and the data from the 24 h DR. Sodium intake (mg/day) was calculated for each participant as a sum of the products of the amount of consumed food or beverage and the sodium content of that specific food or beverage. The discretionary sodium intake was not included in the final estimate of sodium intake.

The aforementioned application allowed for the foods and beverages consumed to be categorized using various descriptors (categorizations). The consumed foods and beverages were divided into the following groups: cereals and cereal-based products, meat and meat products, dairy foods, composite meals, fruits, vegetables and fruit-based and vegetable-based products, sweets, salty snacks, beverages and miscellaneous. Foods and beverages were also categorized into processed foods, composite meals or foods and beverages with natural sodium content (no added sodium). The contribution of each group as well as of specific foodstuffs to the total sodium intake was calculated.

Statistical analysis was performed using the IBM SPSS Statistics version 20.0 (IBM Corporation, 2011). The data were reported as counts and percentages or the mean value ± standard deviation (SD) where applicable. The categorical data were compared using the χ^2-test. The differences between numerical data were assessed using Student's *t*-test.

P values of < 0.05 were considered statistically significant.

Results

A convenience sample of 266 students (mean age 21.26 ± 1.97 years, 45.1% males) both in medical and in non-medical studies participated in the study and completed the 24 h DR. Sample characteristics are shown in Table 1.

Table 1

Sample characteristics (n = 266)

Characteristics	Values
Age (years), $\bar{x}$ ± SD	21.26 ± 1.97
Gender, n (%)	
males	120 (45.1)
females	146 (54.9)
Study program type, n (%)	
medical	42 (15.8)
non-medical	224 (84.2)

$\bar{x}$-mean; SD – standard deviation; n – number of students.

The mean estimated sodium intake of the students was 3,938.5 ± 1,708.1 mg/day (equal to 9.8 ± 4.3 g/day of sodium chloride/salt) (Table 2). The sodium intake of female participants was significantly lower than the sodium intake of male participants ($p < 0.001$). The medical students consu-

Table 2
Mean estimated sodium intake (mg/day) of the students attending the University of Novi Sad (n = 266)

Students	Sodium intake mean ± SD	*t*-value; *p* value
Total sample	3,938.5 ± 1,708.1	
Gender		
males	4,726.5 ± 1,835.1	$t = 7{,}247$; $p < 0.001$
females	3,290.9 ± 1,277.8	
Study program type		
medical	3,029.1 ± 1,035.2	$t = 5{,}449$; $p < 0.001$
non-medical	4,019.1 ± 1,756.5	

SD – standard deviation.

med less sodium *per* day than non-medical students ($p < 0.001$) (Table 2).

The sodium intake of 89.1% of the surveyed students exceeded the WHO guideline for sodium intake. The percentage of male participants consuming more than 2,000 mg of sodium *per* day was higher than the corresponding percentage of female students (95.0% and 84.2%, respectively) ($\chi^2 = 7.841$; $p = 0.005$). There was no statistical difference between the percentage of medical students (83.3%) and the percentage of non-medical students (90.2%) whose sodium intake was above the guideline amount ($\chi^2 = 1.706$; $p = 0.19$).

The food groups that contributed the most to the total sodium intake of the students were meat and meat products (21.7%) and cereals and cereal-based products (18.6%), followed by composite meals (17.0%). The "miscellaneous" category was responsible for 28.7% of the total sodium intake. The contribution of specific food groups to the total sodium intake among the participants is shown in Figures 1, 2 and 3 (total, by gender and by study program type).

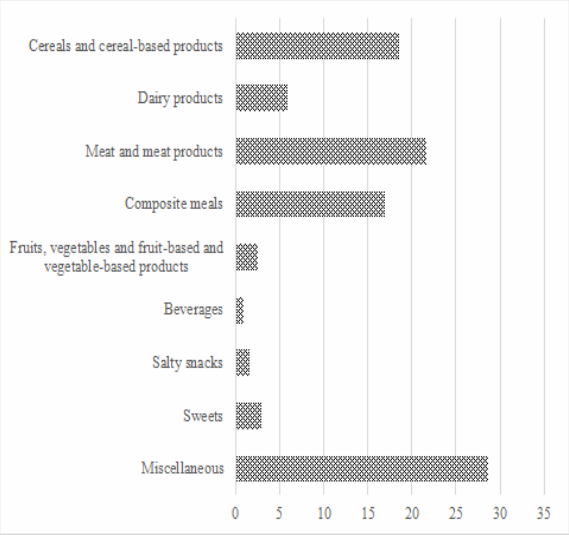

Fig. 1 − The contribution of different food groups to the total sodium intake of students attending the University of Novi Sad (n = 266).

The major dietary sources of sodium among males and females were similar. Males consumed more sodium *via* meat and meat products than females. The non-medical students consumed 17.2% of their total sodium intake through composite meals as opposed to medical students

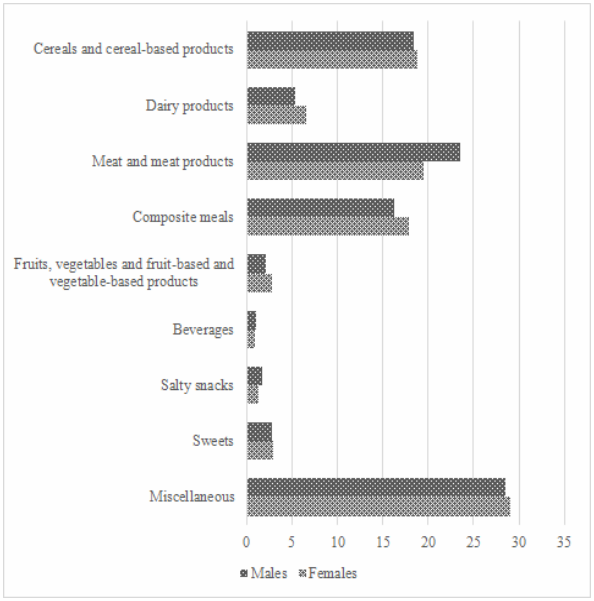

Fig. 2 − The contribution of different food groups to the total sodium intake of the students attending the University of Novi Sad by gender (n = 266).

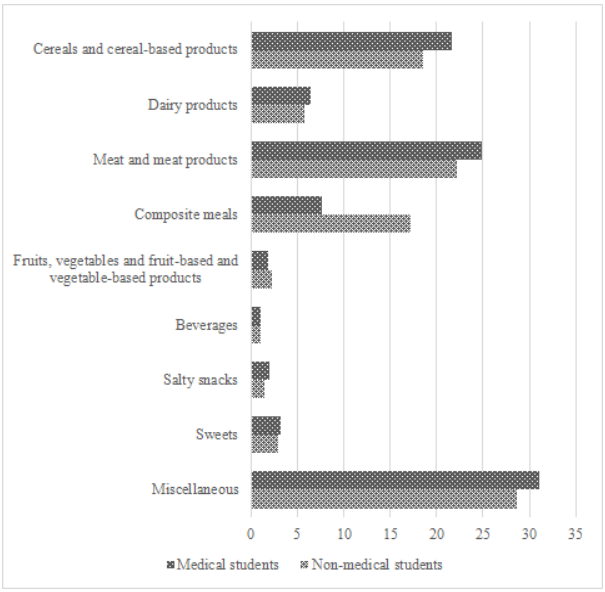

Fig. 3 − The contribution of different food groups to the total sodium intake of the students attending the University of Novi Sad by the study program type (n = 266).

who consumed 7.6% of their total sodium intake by way of composite meals.

The majority of the consumed sodium was from the processed foods (78.9% of the total sodium intake), regardless of the gender and the faculty attended.

Sodium was mostly consumed *via* bread and other bakery products (13.1% of the total sodium intake) and cured meat products (5.1%). Bread alone contributed 9.7% to the total sodium intake or an average of 682.7 mg of sodium/day/person. Almost half of the sodium consumed (49.2%) was from a narrow selection of foods including bread and other bakery products, cured meat products, "fast foods", salty soups, cheeses, sauces and gravies (Table 3).

Table 3

The contribution of specific foods to the total sodium intake of the students attending the University of Novi Sad (n = 266)

Food groups	Contribution of specific foods to the total sodium intake	
	mg sodium/day/person	% of total sodium intake
Bread and other bakery products	746.6	13.1
Cured meat products	1,066.7	8.1
Fast foods (pizza slices, sandwiches, traditionally prepared cheese pastries, traditionally prepared minced meat patties)	1,487.8	14.3
Commercial soups	1,209.7	9.8
Cheese	474.5	2.7
Sauces and gravies (incl. ketchup and mayonnaise)	350.0	1.2
Total		49.2

Discussion

The data presented in this study are the first data on sodium intake of the population of students in Serbia. Undergraduate students were chosen as a sample because they are healthy young adults, as yet unaffected by hypertension or other noncommunicable diseases and among whom risk reduction strategies potentially give better and longer lasting results than among older or hypertensive people.

We found a high sodium intake in the students of the University of Novi Sad (3,938.5 mg/day on the average), that puts them at high risk of developing high blood pressure. The data on sodium intake of the students do not include discretionary sodium added at the table or during cooking and therefore the total sodium intake is most probably underestimated. Discretionary sodium intake usually accounts for 10−15% of the total sodium intake [7, 8, 16]. Nevertheless, the majority of the undergraduate students surveyed in Novi Sad consume more than 2,000 mg of sodium *per* day. The mean estimated sodium intake of the participants is almost double the guideline amount.

Although high, sodium intake of our participants is similar to the reported sodium intakes of other population groups. According to Brinsden and Farrand [5], the average sodium intake worldwide ranges from 2,000−7,200 mg of sodium/day (5 g to 18 g of salt/day). These findings are supported by many authors from different countries [17–24]. The reported sodium intake of people above 2 years of age in the United States (US) is 3,266 mg/day (excluding discretionary sodium intake) [17]. Data from the European Union suggest that sodium intake is in excess of 2,000 mg/day in most European countries [18]. Sodium intake in the general population of Slovenia and Croatia is around 4,500 mg/day [19, 20], while sodium intake in Bulgaria and Hungary is on the average 6,500 mg/day. Sodium intake of our students is lower than the reported sodium intakes in the region probably due to the underestimation of sodium intake in our study, existing differences in the characteristics of the surveyed population groups and possibly due to different methodologies applied.

The female students from our study consume less sodium than males (3,290.9 mg/day and 4,726.5 mg/day, respectively), which is similar to the findings of other authors [6, 18–22, 24, 26, 27]. Gender differences in sodium intake are explained by higher overall food consumption (higher energy intake) among men [6, 26]. Medical students consume less sodium than non-medical students in part due to gender differences – there were more females in the medical students group in comparison to the non-medical students group. Medical students are expected to have better nutrition-related knowledge than non-medical students, which may also be associated with lower sodium intake. One study reported that a daily meal in students dining halls in Novi Sad contained 6,400−7,200 mg of sodium (results from 2008) [28]. The location of students dining halls in Novi Sad, close to the main campus, is inconvenient for medical students (the Faculty of Medicine is the only faculty not located within campus grounds and is considerably for from students dining halls), so the proportion of medical students usually eating in them is not as high as the proportion of non-medical students doing so. This should also be taken into account when comparing sodium intake of medical and non-medical students.

A high sodium content of processed foods is the main reason for excessive sodium intake in the developed "Western" societies [7, 8, 26]. Studies show that up to 77% of sodium consumed in the US [25] and up to 85% of sodium consumed in the United Kingdom is from processed foods [8]. A small proportion of dietary sodium is naturally contained in foods, while the rest of sodium in the diet is added to food during cooking or at the table [8, 26]. Similarly, our results show that 78.9% of the total sodium intake (excluding discretionary sodium intake) of the students from Novi Sad is attributable to processed food consumption. The described results are expected because nutrition transitions are chan-

ging the traditional diets of developing countries, including Serbia, shifting them to diets of cheaper, processed foods, high in saturated fats, added sugars and sodium (added as salt) [29].

Food categories usually contributing the most to the total sodium intake in the US and the European Union are bread, other bakery and cured meat products. Americans consume 7.4% of their sodium through bread and other bakery products and 5.1% of their sodium *via* cured meat products [17], while in Europe percentages vary between countries. In the region, bread and other bakery products were identified as the main source of sodium in the diet of Bulgarians, responsible for 40−50% of the total sodium intake [30]. Bread, bakery products and meat products are also the main sources of sodium in Hungary [30]. Our results show that students consume most of their sodium *via* meat and meat products and cereals and cereal-based products. As in other countries [17, 30, 31], bread and other bakery products (13.1%) and cured meats (5.1%) are significant contributors to the total sodium intake.

It has been reported that in the US, 10 food categories are accountable for 44% of the total sodium intake. We also found that a limited number of food categories (bread and bakery products, cured meat products, "fast foods", salty soups, cheeses, sauces and gravies) are responsible for almost half of the total sodium intake, making it essential that future reformulation efforts on the part of the food industry focus on these food categories. Although the students from our sample consume most of their sodium from foods categorized as "miscellaneous" (28.7%), foods from this group should not be viewed as target foods for sodium reduction efforts (reformulation) due to their diverse nature.

The limitations of this study are: estimation of sodium intake using 24 h DR which may result in underestimation of the total sodium intake (underreporting of the amounts of the foods or not reporting the use of specific foods); exclusion of discretionary sodium intake from the final estimation of the total sodium intake, and the specific (narrow) population group on which the study was conducted, making it hard to extrapolate the results to the general population. Nevertheless, as some of the first data on sodium intake available in Serbia, the results of this study show the need for a more detailed assessment of sodium intake and the sources of sodium in the diet of the Serbian population.

Reduction of salt intake is a complex task and requires the simultaneous involvement of all stakeholders, including government, non-governmental organizations, mass-media, food industry, mass catering industry and consumers [18, 32]. Salt reduction efforts have been reported in some countries in the region, including Slovenia, Hungary, Bulgaria [33] and Croatia [34]. Although the national program for the prevention, medical treatment and control of cardiovascular diseases in Serbia until 2020 ("Healthy Heart for All") [35] mentions a reduction in salt intake in the population as a whole, to date no action has been taken to actively address this issue.

Conclusion

Considering the growing burden presented by hypertension and cardiovascular diseases in Serbia, cost-effective strategies for hypertension and cardiovascular risk reduction, such as sodium intake reduction, should be embraced and actively practiced by policy makers and stakeholders. The labeling of salt content in retail foods should be made obligatory. Food industry should work on reformulating products with high sodium content, primarily bread and bakery products and meat and meat products. Undergraduate students as well as the general population should be empowered to make better dietary and lifestyle choices through education about the health consequences of high sodium intake.

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The usefulness of endoscopic ultrasonography in differentiation between benign and malignant gastric ulcer

Endoskopska ultrasonografija u diferenciranju benignog od malignog ulkusa želuca

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Abstract

Background/Aim. Gastric ulcer may be benign or malignant. In terms of therapy and patient's prognosis early detection of malignancy is very important. The aim of this study was to assess the usefulness of endoscopic ultrasound (EUS) in differentiation between benign and malignant gastric ulcer. **Methods.** A prospective study included 20 consecutive adult patients with malignant gastric ulceration and 20 consecutive adult patients with benign gastric ulceration. All the patients underwent EUS. A total of 6 parameters were analyzed: ulcer width, ulcer depth, the thickness of the gastric wall along the edge of ulceration (T_0), the thickness of the gastric wall 2 cm from the edge of ulceration (T_2), loss of layering structure of the gastric wall, and the presence of regional lymph nodes. EUS criteria for malignancy and a point-score of malignancy were defined. The critical value of total point-score was also calculated showing the best reliability parameters. **Results.** There are 4 criteria for malignancy of gastric ulceration: $T_0 >$ 10 mm, $T_2 >$ 5 mm, EUS visualization of at least one lymph node, loss of layering structure of the gastric wall. Furthermore, $T_2 >$ 5 mm was the only EUS independent predictor of ulcer malignancy. The total point score of ≥ 4 was the cut-off point-score value which gave the best reliability parameters in the assessment of malignant ulcers: sensitivity of 70%, specificity of 95%, positive predictive value of 93.3%, negative predictive value of 76% and accuracy of 82.5%. **Conclusion.** According to the results obtained in this study, we can conclude that EUS is usefull in differentiation between benign and malignant gastric ulcer.

Key words:
endosonography; stomach ulcer; diagnosis, differential; neoplasms.

Apstrakt

Uvod/Cilj. Ulkus želuca može biti benigna ili maligna bolest. Iz aspekta lečenja i prognoze bolesnika, rano otkrivanje maligniteta veoma je važno. Cilj studije bio je da se proceni uloga endoskopskog ultrazvuka (EUS) u diferenciranju benignih od malignih ulkusa želuca. **Metod.** Prospektivnom studijom bilo je obuhvaćeno 20 konsekutivnih odraslih bolesnika sa malignim ulkusom želuca i dvadeset konsekutivnih odraslih bolesnika sa benignim ulkusom želuca. Svim bolesnicima rađena je EUS. Analizirano je šest parametara: širina ulkusa, dubina ulkusa, zadebljanje zida želuca uz ivicu ulceracije (D_0), zadebljanje zida želuca 2 cm od ivice ulceracije (D_2), gubitak slojevne strukture zida želuca i prisustvo regionalnih limfnih čvorova. Formulisani su EUS kriterijumi maligniteta i poen-skor maligniteta. Takođe je proračunata kritična vrednost ukupnog poen-skora, koji je za procenu maligniteta imao najbolje parametre pouzdanosti. **Rezultati.** Postoje četiri kriterijuma maligniteta želudačne ulceracije: $D_0 >$ 10 mm; $D_2 >$ 5 mm, EUS vizualizacija bar jednog limfnog čvora i gubitak slojevne strukture zida želuca. Jedini nezavisni prediktor maligniteta ulceracije bila je vrednost $D_2 >$ 5 mm. Za ukupni poen-skor ≥ 4 dobijeni su najbolji parametri pouzdanosti za procenu maligniteta ulkusa: senzitivnost od 70%, specifičnost od 95%, pozitivna prediktivna stopa od 93,3%, negativna prediktivna stopa od 76% i tačnost od 82,5%. **Zaključak.** Prema rezultatima ove studije može se zaključiti da je metoda EUS korisna za diferenciranje benignih od malignih ulkusa želuca.

Ključne reči:
endosonografija; želudac, ulkus; dijagnoza, diferencijalna; neoplazme.

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Introduction

Gastric ulcer has been defined as a defect in the mucosal surface greater than 5 mm in size, with depth to the submucosa [1]. It may be the consequence of a benign or a malignant disease. In terms of therapy and patient′s prognosis early detection of malignancy is very important. However, the differential diagnosis between benign and malignant ulcers can be difficult. Gastroscopy with biopsy is the most frequently used diagnostic tool. But, sometimes endoscopic appearance of the ulcer does not correlate with the histopathological diagnosis. Repeated biopsies, during the follow up period can show malignant cells even in the case of healed chronic gastric ulcer [2, 3]. Although endoscopic ultrasonography (EUS) is an useful method for gastric wall visualizing, the available data about its role in the differential diagnosis between benign and malignant gastric ulcers are scarce [4].

The aim of this study was to assess the usefulness of EUS in differentiation between benign and malignant gastric ulcer.

Methods

Study design and patient population

A prospective study included 20 consecutive adult patients with malignant gastric ulceration and 20 consecutive adult patients with benign gastric ulceration, treated in our hospital. Informed written consent to participation in the study was obtained from each patient before endosonographic examination. The ethical aspect of this study was approved by the local Ethics Committee.

Diagnosis of gastric ulcer

Gastric ulcer was initially diagnosed by gastroscopy and biopsy. We took four biopsy specimens from ulcer edges and an additional biopsy specimen from the base. Biological nature of ulceration was confirmed histopathologically.

Endosonographic examination

All the patients underwent endosonographic examination using a radial scanning echoendoscope (EU-M30, Olympus) at 7.5 MHz frequency. Examination was preceded by topical oropharyngeal anesthesia. The agitated patients were sedated by intravenously administered midazolam (3–5 mg) or diazepam (5–15 mg). During examination the patients were in the supine position on the left side. A water filled balloon around the transducer was used to provide a fluid interface between the transducer and the gastric wall.

We analyzed 6 endosonographic parameters: ulcer width, ulcer depth, the thickness of the gastric wall along the edge of ulceration (T_0), the thickness of the gastric wall 2 cm from the ulceration edge (T_2), loss of layering structure of the gastric wall, and the presence of regional lymph nodes. Loss of layering structure of the gastric wall was defined as discontinuity in the layers of the gastric wall below the mucosa.

Each of the 6 endosonographic parameters was compared between the groups with benign and malignant ulcers and those showing a statistically significant difference were considered the EUS criteria of malignancy. Independent predictors of malignancy were defined, as well.

Finally, we made a point-score of malignancy, so what we scored was the existence of EUS criteria of malignancy with two points (for independent predictors) or one point (for other EUS criteria of malignancy). The points were tallied and a total point score of malignancy was defined. The critical value of total point-score was also calculated showing the best reliability parameters: sensitivity, specificity, positive predictive value, negative predictive value and accuracy.

Statistical analysis

Data processing was performed using SPSS 11.5 for Windows software (SPSS, Inc., Chicago, IL). Average values were presented as mean value ± standard deviation, and a *p*-value of 0.05 (two-sided) was considered to be statistically significant. Two sets of parametric data were compared by the unpaired Student's *t*-test and two sets of categorical data were compared by the Mann-Whitney's *U*-test and the Pearson's (χ^2) test. Binary logistic regression analysis was performed to define EUS criteria of malignancy, and Forwald: Wald multivariate logistic regression analysis was used to define independent predictors of malignancy.

Results

Characteristics of the patients

Benign gastric ulcer was diagnosed in 11 males and 9 females (average age 65.7 ± 10.4 years). Malignat gastrisc ulcer was diagnosed in 13 males and 7 females (average age 59.5 ± 13.5 years). The lesser curvature was the most frequent ulcer site, both for benign (60%) and malignant ulcers (50%).

Ulcer width and ulcer depth

Ulcer width ranged 4.2–30 mm for benign and 3–35 mm for malignant ulceration and ulcer depth ranged 2–17 mm for benign and 2–15 mm for malignant ulcerations (Tables 1 and 2). In relation to benign ulcers, malignant ulcers were wider in average (16.3 ± 7.5 mm and 14.6 ± 7.2 mm, respectively) and deeper in average (7.4 ± 4.1 mm and 5.4 ± 3.7 mm, respectively), but the differences were not statistically significant (unpaired Student's *t*-test, $p > 0.05$) (Table 3).

T_0 ranged 4–17 mm for benign and 3–24.5 mm for malignant ulcerations (Tables 1 and 2). In average, To was significantly greater in malignant ulcer group than in benign ulcer group (13.2 ± 5.8 mm and 9.1 ± 3.2 mm; $p = 0.009$; unpaired Student's *t*-test) (Table 3). Thickness of 10 mm was defined as a cut-off value which provided maximum statistical significance in differentiation between benign and malignant ulcers. That was proved both by the Mann-Whitney *U*-test ($p = 0.001$; $Z = -3.138$) and the Pearson's (χ^2) test ($p = 0.001$; $\chi^2 = 10.101$).

Table 1

Endosonographic parameters and endosonographic criteria of malignancy in the patients with benign ulcers

Patient No	Endosonographic parameters						EUS criteria of malignancy				Point score
	U_w (mm)	U_d (mm)	T_0 (mm)	T_2 (mm)	LLS	LN presence	A	B	C	D	Σ
1	27	17	17	10	+	+	1	**2**	1	1	5
2	25	9	10	10	+	-	-	**2**	-	1	3
3	9	3	7	3	-	+	-	-	1	-	1
4	10	2	8	3	+	-	-	-	-	1	1
5	20	9.6	16.6	2.7	+	-	1	-	-	1	2
6	13	4	9	5	+	-	-	-	-	1	1
7	12.1	4.5	13.1	4.2	-	-	1	-	-	-	1
8	4.2	2	7.2	3.2	+	-	-	-	-	1	1
9	19	9	4	3	-	+	-	-	1	-	1
10	17	6.1	6	4	-	-	-	-	-	-	0
11	5.5	2.5	7.6	3.6	-	-	-	-	-	-	0
12	16.2	6.5	7.3	3.3	+	-	-	-	-	1	1
13	10	2.3	8.3	3.1	-	-	-	-	-	-	0
14	17	4	7	3	-	-	-	-	-	-	0
15	10	5	9	5	+	-	-	-	-	1	1
16	16.5	4.5	9.4	4.7	+	+	-	-	1	1	2
17	17	4	7	4	+	-	-	-	-	1	1
18	6.9	3.1	8.4	3.3	+	-	-	-	-	1	1
19	7.4	2	10	3.6	-	-	-	-	-	-	0
20	30	8	11	5	+	-	1	-	-	1	2

Uw – ulcer width; Ud – ulcer depth; T_0 – gastric wall thickness along the edge of ulceration; T_2 – gastric wall thickness 2 cm from the ulceration edge; LLS – loss of layering structure of the gastric wall; LN – lymph nodes; EUS – endosonographic ultrasound; A ⇒ T_0 > 10 mm; B ⇒ T_2 > 5 mm; C ⇒ EUS visualization of at least 1 lymph node; D ⇒ loss of layering structure of the gastric wall.

Table 2

Endosonographic parameters and endosonographic criteria of malignancy in patients with malignant ulcers

Patient No	Edosonographic parameters						EUS criteria of malignancy				Point score
	U_w (mm)	U_d (mm)	T_0 (mm)	T_2 (mm)	LLS	LN presence	A	B	C	D	Σ
1	8	2	4.1	3	-	-	-	-	-	-	0
2	10	10	3	3	+	+	-	-	1	1	2
3	8	4	18.9	10	+	+	1	2	1	1	5
4	11.4	15	14	4	+	-	1	-	-	1	2
5	19.1	8.3	9.6	5.2	+	+	-	2	1	1	4
6	26.7	13.9	22.2	7	+	+	1	2	1	1	5
7	14.4	8.3	13.8	12.3	+	+	1	2	1	1	5
8	16.7	3.6	8.4	8.8	+	+	-	2	1	1	4
9	23.2	9.2	15.3	4.1	+	+	1	-	1	1	3
10	15.7	6.5	15	5.7	+	+	1	2	1	1	5
11	16.8	12	14.9	8	+	+	1	2	1	1	5
12	10	4	15	15	+	+	1	2	1	1	5
13	20	15	18	15	+	+	1	2	1	1	5
14	14.2	6.6	8.2	3	+	-	-	-	-	1	1
15	3	2	3.5	3	-	-	-	-	-	-	0
16	22	8	16	20	+	-	1	2	-	1	4
17	22	6	24.5	10	+	+	1	2	1	1	5
18	10	2	15.8	7.8	+	-	1	2	-	1	4
19	35	5	12.5	20.3	+	+	1	2	1	1	5
20	20.6	6	12	8	+	-	1	2	-	1	4

Uw – ulcer width; Ud – ulcer depth; T_0 – gastric wall thickness along the edge of ulceration; T_2 – gastric wall thickness 2 cm from the ulceration edge; LLS – loss of layering structure of the gastric wall; LN – lymph nodes; EUS – endosonographic ultrasound; A ⇒ T_0 > 10 mm; B ⇒ T_2 > 5 mm; C ⇒ EUS visualization of at least 1 lymph node; D ⇒ loss of layering structure of the gastric wall.

Table 3

Endosonographic parameters in patients with gastric ulceration in our series

Endosonographic parameters	Benign ulceration	Malignant ulceration	*p*	Statistical parameters
Ulcer width (mm), $\bar{x} \pm SD$	14.6 ± 7.2	16.3 ± 7.5	> 0.05[a]	
Ulcer depth (mm), $\bar{x} \pm SD$	5.4 ± 3.7	7.4 ± 4.1	> 0.05[a]	
T_0 (mm), $\bar{x} \pm SD$	9.1 ± 3.2	13.2 ± 5.8	0.009[a]	t = -2.755 CI = -7.0957/-1.0843
T_2 (mm), $\bar{x} \pm SD$	4.3 ± 2.1	8.7 ± 5.4	0.002[a]	t = -3.336 CI = -6.9498/-1.7002
LLS, n	12	18	0.031[b]	Z^b =-2.163 χ^2c = 4.800 (DF = 1)
Visualisation of lymph node, n	4	13	0.004[b]	Z^b = -2.842 χ^2c = 8.286 (DF = 1)

T_0 – gastric wall thickness along the edge of ulceration; T_2 – gastric wall thickness 2 cm from the ulceration edge; LLS – loss of layering structure of the gastric wall; $\bar{x}$ – mean; SD – standard deviation; n – number of patients; [a]unpaired-samples Student's *t*-test (DF = 38). [b]Mann-Whitney *U*-test; [c]χ^2 test; CI – confidence interval; $p \leq 0.05$ considered to be statistically significant.

T_2 ranged 2.7–10 mm for benign and 3–20.3 mm for malignant ulcerations (Tables 1 and 2). In average, T_2 was significantly greater in the malignant ulcer group than in the benign ulcer group (8.7 ± 5.4 mm and 4.3 ± 2.1 mm; p = 0.002, unpaired Student's *t*-test) (Table 3). Thickness of 5 mm was defined as a cut-off value which provided a maximum statistical significance in differentiation between benign and malignant ulcers. That was proved both by the Mann Whitney *U*-test ($p < 0.001$; Z = -3.824) and the Pearson's (χ^2) test ($p < 0.001$; $\chi^2 = 15.000$).

Loss of layering structure of the gastric wall existed in 18 of 20 (90%) malignant and in 12 of 20 (60%) benign ulceration and the difference was statistically significant (Mann-Whitney *U*-test and Pearson's χ^2 test; $p = 0.031$) (Table 3).

Lymph nodes

Lymph nodes were found in 4 of 20 (20%) benign and in 13 of 20 (65%) malignant ulcerations, and this difference was statistically significant (Mann Whitney *U*-test and Pearson's χ^2 test ; $p = 0.004$) (Table 3). The number of endosonographically seen lymph nodes was 1–2 (1.5 ± 0.5 in average) for benign ulcer group and 1–7 (3.5 ± 2.2 in average) for the malignant ulcer group. All endosonographically seen lymph nodes were with round shape and hypoechoic features, regardless of whether it was malignant or benign ulceration. Furthermore, clear outer border existed in all lymph nodes, seen by endosonographic examination of malignant ulcerations, and in 2 out of 4 patients with benign ulcer. There was the diameter of lymph node greater than 10 mm in 84.6% (11 of 13) of malignant ulcerations and in 25% (1 of 4) of benign ulcerations.

EUS criteria for ulcer malignancy

Binary logistic analysis revealed 4 significant criteria for malignancy of gastric ulceration: A $\Rightarrow$ $T_0 > 10$ mm; B $\Rightarrow$ $T_2 > 5$ mm; C $\Rightarrow$ EUS visualization of at least one lymph node; D $\Rightarrow$ Loss of layering structure of the gastric wall (Table 4). Furtherrmore, $T_2 > 5$ mm was the only EUS independent predictor of ulcer malignancy (Forwald: Wald multivariate logistic regression analysis; $p = 0.001$; RR = 0.048; CI = 0.008/0.273).

Point-score of malignancy

Maximal total point-score of malignancy was 5 points (2 points for independent predictor and 1 point for other 3 EUS criteria for ulcer malignancy). There was a total point score of ≥ 4 in 14 malignant and in only 1 benign ulceration (Tables 1 and 2). This was the cut-off point-score value which gave the best reliability parameters in the assessment of malignant ulcers: sensitivity of 70%, specificity of 95%, positive predictive value of 93.3%, negative predictive value of 76% and accuracy of 82.5%.

Discussion

Over the last 25 years EUS has become accurate diagnostic and therapeutic procedure. It is usefull for locoregional staging of primary gastric, esophageal and rectal cancer and for the diagnosis of gastric subepithelial lesions smaller than 2 cm and small pancreatic tumors [5–10]. It is also recommended for the diagnosis of choledocholithiasis due to smaller number of complications compared to endoscopic retrograde cholongiopancreatography [11]. Therapeutic interventions such as drainage of peripancreatic fluid collections, pancreatic cyst ablation and celiac plexus neurolysis can be performed under linear endoscopic ultrasound guidance [12, 13]. Furthermore, recent data show the role of EUS in creating bilioenteric and enteroenteric anastomosis in selective situations [12].

The role of EUS in the differential diagnosis of benign and malignant ulcers has been poorly investigated. The main method for differentiating benign from malignant ulcer is still gastroscopy with biopsy, but sometimes, gastric carcinoma endoscopically may look like a benign ulcer. Moreover endoscopic biopsies do not always confirm malignancy, leading to delays in correct diagnosis. Some authors suggest "that the location and diameter of gastric ulcers may be used as a marker of risk factors for developing gastric cancer" [14]. According to our results, the difference in the width and depth of ulceration, between malignant and benign ulcers was not statistically significant. In the study by Lv et al. [15], biopsies from the ulcer bases and edges at the repeated endoscopies, have shown better results in detection of gastric cancer in comparasion with the biopsies taken from the edges of ulcers only [15]. Some studies demonstrated that magnifying endoscopy with narrow-band imaging has an advantage in comparison with conventional endoscopy for differential diagnosis of a small depressed gastric lesion [16, 17], while chromoendoscopy has not improved the differentiation of gastric ulcers with respect to their origin [18]. We found different data about the usefulness of computed tomography (CT) virtual gastrocopy. According to the results of Mochetta et al. [19] study, CT virtual gastroscopy is superior to conventional endoscopy and allows differentiation between benign and malignant gastric ulcer based on morpho-

Table 4

Endosonographic ultrasound criteria for ulcer malignancy in the patients with gastric ulceration (binary logistic analysis)

Endosonographic criteria	Benign ulceration	Malignant ulceration	*p*	Statistical parameters
$T_0 > 10$ mm	4	14	0.003	RR = 0.107 CI = 0.025/0.459
$T_2 > 5$ mm	2	14	0.001	RR = 0.048 CI = 0.008/0.273
Loss of layering structure of the gastric wall	12	18	0.04	RR = 0.167 CI = 0.030/0.924
Visualisation of lymph node	4	13	0.006	RR = 0.135 CI = 0.032/0.562

T_0 – gastric wall thickness along the edge of ulceration; T_2 – gastric wall thickness 2 cm from the ulceration edge; RR – relative risk, CI – confidence interval; $p \leq 0.05$ considered to be statistically significant.

logical features. In the study by Chen et al. [20] virtual gastroscopy and classic endoscopy "were almost equally useful" in differentiation between malignant and benign gastric ulcers.

In contrast to aforementioned diagnostic procedures, EUS allows visualization of the gastric wall, but this advantage was rarely used for differential diagnosis of gastric ulcers. Both types of ulcers may share the same basic endosonography findings: low echo mass (originated from fibrosis and granulation), symmetrical or asymmetrical convergence of the submucosal layer, thickening of the gastric wall, making diagnosis difficult [21, 22]. Zhang et al. [4] formulated the following characteristics of malignant gastric ulcer: unclear architectures, hypoechoic mass with partial or total destruction of the normal wall architecture and malignant lymph nodes.

According to our study there are 4 significant criteria of malignancy of gastric ulceration: $T_0 > 10$ mm; $T_2 > 5$ mm; EUS visualization of at least one lymph node and the loss of layering structure of the gastric wall.

In our patients gastric wall thickness was significantly higher in the malignant ulcer group than in the benign ulcer group. It is known that the thickened gastric wall in patients with benign gastric ulcer correlates well with the spread of fibrosis [21]. The majority of authors agree that in cases of ulcerative cancers the thickening of the gastric wall correlates better with the spread of fibrosis, than with cancer invasion, which leads to overestimation of the depth of invasion [22–26]. In the available literature we were unable to find any data on the significance of the wall thickness in the differential diagnosis between benign and malignant gastric ulcer. According to our results the values greater than 10 mm and 5 mm for T_0 and T_2 respectively, were defined as criteria of malignancy. Moreover, T2 greater than 5 mm was found as the only EUS independent predictor of ulcer malignancy. Interestingly, the maximum thickness of the gastric wall we found in patients with malignant lymphoma. Among 3 of the patients with malignant lymphoma, T_0 was greater than 20 mm in 2 of the patients, while T_2 was greater than 20 mm in 1 patient.

Our results show that EUS visualization of at least 1 lymphe node significantly increases the likelihood of ulcer malignancy. All endosonographically seen lymph nodes were with round shape and hypoechoic features, regardless of whether it was malignant or benign ulceration. Because of that, shape and echogenicity of lymph node are not a reliable criterion for differentiating benign from malignant ulcers. Valid statistical analysis of the differences between benign and malignant ulcers based on the diameter of a lymph node and the clarity of its outer border was impossible because of a small sample in benign ulcers. In the study of Gill et al. [27] round shape, a short axis greater than 8.3 mm, and sharp margins were predictive of the involvement of lymph node by metastasis. Similar results were published by some other authors [28, 29]. On the other side, Jamil et al. [30] poented out that echo feature of lymphe node is not a reliable sign of malignancy and they suggest fine needle aspiration under endoscopic ultrasound guidance.

Histopathological examinations of gastric wall and lymph nodes were done in 14 of the patients with malignant ulcerations, who underwent gastric surgery. Endosonographic assessment of T stage coincided with the postoperative histopathological findings in 12 of 14 (85.7%) operated patients. The T stage was underestimated in 2 of the patients. Endosonographic assessment of the N stage coincided with the postoperative histopathological findings in 9 of 14 (64.3%) operated patients. The N-stage was underestimated in 4 of the patients and overestimated in 1 patient.

Loss of layering structure of the gastric wall may exists in both types of ulcerations, due to the spread of fibrosis or cancerous tissue below the mucosa [21, 24]. According to our results, there was the loss of layering structure of the gastric wall in 90% of ulcerative carcinoma. This parameter was incuded in the criteria of malignancy in the study by Zhang et al. [4].

Combining EUS criteria of malignancy increases the reliability in defining the biological nature of gastric ulceration. Assessment of malignancy of gastric ulcers based on 4 EUS criteria and point-score, which were formulated in our study, is fast and reliable. In comparasion with the results of Zhang et al. [4], only the sensitivity of EUS for diagnosis of malignancy in our study was lower: 75% *vs* 83.8% [4]. However, further experience gained on larger series of patients will be the best way to check the reliability of these criteria and possibly to correct them.

Conclusion

Our results point to the usefulness of endosonographic ultrasound in differentiation benign from malignant gastric ulcer. It is very important, especially in case of disagreement between the endoscopic appearance of ulcerations and histological findings.

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The distribution of genetic polymorphism of CYP3A5, CYP3A4 and ABCB1 in patients subjected to renal transplantation

Distribucija polimorfizma gena koji kodiraju CYP3A5, CYP3A4 i P-glikoprotein kod bolesnika podvrgnutih transplantaciji bubrega

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Abstract

Background/Aim. Polymorphisms of genes which encode transporter P-glycoprotein and most important enzymes for tacrolimus pharmacokinetics can have significant influence reflecting on blood concentrations of this drug. The aim of this study was to examine the distribution of polymorphisms of CYP3A5, CYP3A4 and ABCB1 genes in patients subjected to renal transplantation, for the first time in our transplantation center. **Methods.** The research was designed as a prospective cross-sectional study which included 211 patients subjected to renal transplantation in the Centre for Solid Organ Transplantation of the university tertiary health care hospital, Military Medical Academy, Belgrade, Serbia. Patients of both genders, 22−69-year-old, Caucasians, subjected to immunosuppressive regimen, including tacrolimus, were recruited for the study. CYP3A5 6986A>G (the *3 or *1, rs776746), CYP3A4 -392A>G (the *1 or *1B, rs2740574) and ABCB1 3435C>T (rs1045642) genotypes were determined by TaqMan® SNP genotyping assays. **Results.** Most of our patients (94.8%) had functional CYP3A4 enzyme, while 87.7% of all the patients had diminished CYP3A5 enzymatic activity. On the other hand, about one third of them, 31.3%, had functional ABCB1 transporter. **Conclusion.** A total of 84.8% of our patients were found to express both the CYP3A5*3*3 genotype (associated with diminished CYP3A5 enzymatic activity) and CYP3A4*1*1/*1*1B (associated with functional CYP3A4 enzymatic activity), while out of all the patients with diminished CYP3A5 enzymatic activity, 68.7% had diminished activity of ABCB1 transporter. However, further studies are necessary in order to show the influence of these genetic polymorphisms on tacrolimus blood concentrations in patients after renal transplantation.

Key words:
tacrolimus; kidney transplantation; polymorphism, genetic; cyp3a4 protein, human; cyp3a5 protein, human; abcb1 protein, human.

Apstrakt

Uvod/Cilj. Polimorfizmi gena koji kodiraju transporter P-glikoprotein i najvažnije enzime za farmakokinetiku takrolimusa mogu imati značajan uticaj koji se odražava na koncentracije ovog leka u krvi. Cilj ovog ispitivanja bio je da se ispita učestalost polimorfizma gena CYP3A5, CYP3A4 i ABCB1 kod bolesnika sa transplantiranim bubregom, po prvi put u našem centru za transplantaciju. **Metode.** Urađena je prospektivna studija preseka koja je obuhvatila 211 bolesnika sa urađenom transplantacijom bubrega u Centru za transplantaciju solidnih organa Vojnomedicinske akademije u Beogradu. U studiju su bili uključeni bolesnici oba pola, starosti od 22 do 69 godina, bele rase i na imunosupresivnom režimu koji uključuje takrolimus. Urađena je genotipizacija CYP3A5 6986A>G (*3 ili *1, rs776746), CYP3A4 -392A>G (*1 ili *1B, rs2740574) i ABCB1 3435C>T (rs1045642) korišćenjem TaqMan® eseja za određivanje pojedinačnih nukleotidnih polimorfizama. **Rezultati.** Većina naših bolesnika (94,8%) imala je funkcionalan CYP3A4 enzim, dok je kod 87,7% od svih naših bolesnika CYP3A5 bio sa oštećenom aktivnošću. S druge strane, kod oko trećine (31,3%) bolesnika ABCB1 transporter bio je funkcionalan. **Zaključak.** Ukupno 84,8%

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naših bolesnika imalo je istovremeno CYP3A5*3*3 genotip, povezan sa smanjenom CYP3A5 aktivnošću, i CYP3A4*1*1/*1*1B genotip, povezan sa funkcionalnom formom CYP3A4 enzima, dok je od svih bolesnika sa smanjenom CYPA5 enzimskom aktivnošću 68,7% imalo smanjenu aktivnost ABCB1 transportera. Međutim, buduće studije su neophodne kako bi se pokazao uticaj ovih genskih polimorfizama na koncentraciju takrolimusa u krvi bolesnika nakon transplantacije bubrega.

Ključne reči:
takrolimus; transplantacija bubrega; polimorfizam, genetički; cyp3a4 protein, humani; cyp3a5 protein, humani; abcb1 protein, humani.

Introduction

Kidney transplantation presents the best way of treating patients with end-stage renal disease. The success of kidney transplantation depends on a delicate balance between the level of immunosuppression, graft rejection and occurrence of adverse effects of immunosuppressive drugs [1, 2].

Tacrolimus (Tac) is one of the most important immunosuppressive drugs which significantly improve the results of kidney transplantation [3]. However, despite its long-standing and wide applications, there are still difficulties in the optimal dosing of this drug due to significant inter- and intraindividual variability of Tac [4]. Among numerous factors that have been identified as contributors to Tac variability, bioavailability of the drug is a prominent one [5, 6]. The bioavailability may largely be due to the presence of genetic polymorphisms which are responsible for synthesis of the enzymes and transporter P-glycoprotein involved in the pharmacokinetics of the drug. It is believed that in the general population genetic is responsible for 20−95% of variability of Tac bioavailability [7]. Recently, there has been a great interest in determination of genetic polymorphisms which could predict a degree of Tac bioavailability in each patient individually [6]. Identification of genetic polymorphisms of enzymes and P-glycoprotein is a rather attractive option for individualized and efficient implementation of Tac in clinical practice. This is more important if we take into account that the genotype is stable and the immutable characteristic needs to be determined only once for a particular gene [2, 7]. Currently, the prospective clinical studies need to demonstrate if determinations of genetic polymorphisms for enzymes and the transporter involved in the pharmacokinetics of Tac before transplantation may contribute to better efficiency and safety of this drug after kidney transplantation.

Since several studies have already shown that differences in the expression of certain genes that affect Tac pharmacokinetics may result in large and unexpected variations in Tac blood concentrations in patients after the equal dose of this drug, the aim of this study was to examine the distribution of polymorphisms of CYP3A5, CYP3A4 and ABCB1 genes in patients subjected to renal transplantation, for the first time in our transplantation center.

Methods

This prospective cross-sectional study included 211 patients, 136 (64.45%) men, and 75 (35.55%) women, subjected to kidney transplantation and follow-up procedures in the Center for Transplantation of Solid Organs in the Military Medical Academy (MMA), Belgrade, Serbia. All the patients were Caucasians, aged from 22 to 69 (median 45). The patients received triple immunosuppression regimen including Tac, mycophenolate mofetil and prednisone, in usual doses [8, 9].

Informed consent was obtained from all the patients. The study was approved by the Ethics Committee of MMA (Ethical approval N° 01/31-01-13, the study protocol N° 910-1).

Peripheral blood was collected in EDTA tubes and stored at -40°C. DNA was extracted from blood by a Pure Link™ Genomic DNA Mini Kit (Invitrogen, USA) according to manufacturer's instructions.

The 211 adult patients were genotyped for single nucleotide polymorphism (SNP) of CYP3A5 at position 6986A>G (the *3 or *1, rs776746), CYP3A4 at position -392A>G (the *1 or *1B, rs2740574) and ABCB1 at exon 26 (3435C>T, rs1045642). The genotyping was detected by TaqMan® SNP genotyping assays (Life Technologies, USA) on a 7500 Real-Time PCR System (Applied Biosystems, USA).

For CYP3A4, ABCB1 and CYP3A5, the observed genotype (allele) frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$).

Data statistical analysis was done using the statistical software package, IBM SPSS Statistics version 19. All variables were presented as a frequency of certain categories.

Results

In the present study, 187/211 (87.7%) patients with kidney allograft were homozygous carriers of the variant CYP3A5*3 allele. Wild type CYP3A5*1 (homozygous or heterozygous carriers) allele was found in 26/211 (12.3%) patients. The CYP3A4 genotyping showed that the majority of patients (210/211; 95.5%) had the wild type *1 allele as homozygous or heterozygous carriers of this variant. Analysis of ABCB1 showed that 66/211 (31.3%) patients were CC homozygote, 92/211 (43.6%) were CT heterozygote and 53 (25.1%) were homozygous carriers of the variant T allele. The frequencies of CYP3A5, CYP3A4 and ABCB1 genotypes are summarized in Table 1.

The results of the present study revealed that the majority of patients were found to express both CYP3A4*1*1/*1*1B genotype, associated with functional CYP3A4 enzymatic activity, and the CYP3A5*3*3 genotype, associated with diminished CYP3A5 enzymatic activity. However, six patients had both CYP3A4*1B and CYP3A5*3 genotype, associated with diminished CYP3A5 and CYP3A4 enzymatic activity (Table 2). Considering CYP3A5 and ABCB1, the majority of patients (68.7%) had diminished both CYP3A5 enzymatic activity and ABCB1 transporter activity (homozygous and heterozygous carriers of the variant T allele) (Table 3).

Table 1

Distribution of genetic polymorphisms significant for tacrolimus pharmacokinetics in the patients subjected to renal transplantation

Gene	Genotype	n (%)
CYP3A5 6986	AA (*1*1)	1 (0.5)
	AG (*1*3)	25 (11.8)
	GG (*3*3)	185 (87.7)
CYP3A4 -392	AA (*1*1)	200 (94.8)
	AG (*1*1B)	10 (4.7)
	GG (*1B*1B)	1 (0.5)
ABCB1 3435	CC	66 (31.3)
	CT	92 (43.6)
	TT	53 (25.1)

Table 2

Distribution of genetic polymorphisms encoding CYP3A4 and CYP3A5 enzymes in renal transplant recipients

Gene	Genotype	CYP3A4 -392A>G, n (%)		
		AA (*1*1)	AG (*1B)	GG (*1B)
CYP3A5 6986A>G	AA (*1*1)	1 (0.5)	-	-
	AG (*1*3)	20 (9.5)	4 (1.9)	1 (0.5)
	GG (*3*3)	179 (84.8)	6 (2.8)	-

Table 3

The distribution of genetic polymorphisms encoding enzyme CYP3A5 and transporter P-glycoprotein in the patients subjected to renal transplantation

Gene	Genotype	ABCB1 3435C>T, n (%)		
		CC	CT	TT
CYP3A5 6986A>G	AA (*1*1)	-	-	1 (0.5)
	AG (*1*3)	5 (2.4)	14 (6.6)	6 (2.8)
	GG (*3*3)	61 (31.3)	78 (43.6)	46 (25.1)

Discussion

The genes that are primarily involved in the pharmacokinetics of Tac are those encoding CYP3A family of enzymes and P-glycoprotein. Tacrolimus undergo substantial intestinal and liver metabolism after absorption from the gut lumen, and it is believed that CYP3A and P-glycoprotein are largely responsible for poor oral bioavailability of this drug [10]. The most important enzymes for Tac metabolism which belong to CYP3A family are CYP3A5 and CYP3A4. Since polymorphic CYP enzyme family is the most important system involved in Tac biotransformation, genotyping of these CYP polymorphisms provides important information that can predict its bioavailability.

While functional CYP3A4 is located in the liver and small intestine of each individual, functionally active CYP3A5 only exists in some individuals ("CYP3A5 expressers"). In our study 12.3% of patients were CYP3A5 expressers. Expressers have at least one wild-type allele (CYP3A5*1), and carry CYP3A5*1*1 or CYP3A5*1*3 genotype. On the other hand, 87.7% of our patients had CYP3A5*3*3 genotype. According to Provenzani et al. [2], it means that they are "CYP3A5 non-expressers" since they are homozygote for mutant allele CYP3A5*3. Homozygous carriers of CYP3A5*3 do not express the enzyme due to creation of a cryptic splice site [11]. Almost all studies have confirmed that carriers of CYP3A5*3*3 genotype require lower doses of Tac than carriers of CYP3A5*1*1 and CYP3A5*1*3 genotype in order to maintain drug level in optimal range [12]. On the other hand, CYP3A5*1 carriers, from all ethnic groups, had around 1.5−2 fold lower blood concentrations for a given dose of Tac than CYP3A5*3 homozygote, what could result in serious therapeutic failure and early acute rejection episodes [13]. We consider that it can be said that CYP3A5 expressers need higher Tac doses, rather that CYP3A5 non-expressers need lower doses of this drug.

Our results are also in accordance with the ones reported by other authors who have examined the frequency of this polymorphism in different ethnic groups [14]. The frequency of CYP3A5 variant alleles shows significant interethnic differences, with the wild-type CYP3A5*1 allele more common in Africans than in Caucasians and Asians [15]. Namely, percentage of non-expresser state occurs in 85% to 95% Caucasians, while in African-Americans this ratio is reversed [14]. Thus, the empirical observation that patients of African-American race required higher Tac doses in order to achieve the same blood concentration in comparison to Caucasians received its theoretical explanation [16].

CYP3A4 is the major cytochrome P450 isoform present in adult liver. There is a large inter-individual variability in hepatic CYP3A4 expression [17], since more than 20 mutations in the CYP3A4 gene have been identified, most of them with unclear clinical importance so far. Some recent studies have demonstrated that primary CYP3A4 polymorphism impli-

cated in Tac metabolism was A > G substitution at position -392 that produced variant allele referred as CYP3A4*1B with diminished enzymatic activity and reduced Tac clearance [14]. Therefore, pre-transplantation genotyping of the CYP3A4*1B, along with CYP3A5*3, could potentially bring benefit to the patients by reducing initial Tac doses among CYP3A poor metabolizers and thereby reduce the risk of reaching over therapeutic Tac concentrations [18].

Most of our patients (95.5%) were homozygous or heterozygous carriers of the variant CYP3A4*1, which were associated with the functional state of the enzyme CYP3A4. The other authors also have pointed out that enzyme CYP3A4 is predominantly active in Caucasians, in whom the presence of this polymorphism ranges from 90−98% [14, 19].

If we consider CYP3A5*3 and CYP3A4*1B polymorphism together, most of our patients (84.7%) were characterized by functional form of the CYP3A4 enzyme and diminished CYP3A5 enzymatic activity. However, since six patients had both diminished enzymatic activities of these enzymes, overexposure of them to Tac could have been expected, if the usual doses of drug were used.

Regarding ABCB1 3435C > T polymorphism alone, over 68% of patients in our study had variant T allele (CT or TT genotype), which was associated with diminished activity of P-glycoprotein. P-glycoprotein, which is encoded by the ABCB1 gene, is a large ATP-dependent transmembrane protein involved in the extracellular extrusion of many drugs, including Tac [10]. More than 700 variations in the nucleotide sequences of ABCB1 gene have been described, and some of them seem to influence the pharmacokinetics of Tac. The most extensively investigated single nucleotide polymorphisms of ABCB1 were 3435 C > T (rs1045642) in exon 26, 1236 C > T (rs128503) in exon 12, and 2677 G>T/A (rs2032582) in exon 21 [20]. It was shown that patients with wild-type genotype ABCB1 (CC) had stable Tac blood concentrations, while patients with CT or TT genotypes had up to 60% higher Tac blood levels [21]. However, many authors showed that genetic polymorphisms for P-glycoprotein *per se* had no major clinical impact on Tac metabolism, except when it existed in combination with genetic polymorphisms for CYP3A5, such as CYP3A5*3 [22]. Therefore, the effect of ABCB1 polymorphisms should be best estimated considering its association with CYP3A5 non expressers and CYP3A5 expresser's status separately [21].

Considering CYP3A5 and ABCB1 polymorphism together, most of our patients (68.7%) had CYP3A5*3*3 genotypes, associated with diminished CYP3A5 enzymatic activity, and diminished activity of ABCB1 transporter (CT or TT genotypes). Overexposure of these patients to Tac can also be expected.

In addition to these genetic polymorphisms, some authors have found that genetic polymorphisms of P450 oxidoreductase (POR) *28 and CYP3A4*22 may potentially influence Tac pharmacokinetics in patients subjected to renal transplantation [2], as well as CYP3A5 polymorphism [23].

Namely, POR is essential for the electron donation in the microsomal-CYP450-mediated mono-oxygenation, and about 40 SNPs have been identified in the POR gene [2]. POR*28 C > T mutations can increase activity of this enzyme and alter the baseline metabolic capacity of several CYP isoforms. The Tac bioavailability in patients who were CYP3A5 expressers, as well as carriers of the wild type CC POR genotype was higher than that observed in patients carrying POR allelic variants. No significant differences were observed between POR*28 CC homozygote and POR*28 T carriers in CYP3A5 non-expressers [24, 25]. Therefore, the POR genotype is important in influencing Tac metabolism only in CYP3A5 expressers.

On the other hand, CYP3A4*22 allele (rs35599367 C > T in intron 6) can also have significant influence on the Tac pharmacokinetics in renal transplant recipients [2]. The patients carrying one or two T alleles required significantly lower Tac doses comparing with the patients who were homozygous for the wild-type C allele [18, 26]. This CYP3A4*22 SNP is significantly linked to reductions in CYP3A4 mRNA production and enzyme activity in human livers [18].

Finally, it appears that CYP3A5 genotyping both in organ recipient and donor is very important for establishing personalized Tac dosage regimen. Namely, CYP3A5*3 polymorphism both in pediatric liver recipients and donors has influence on Tac dosing requirement [23]. Therefore, it was suggested that early determination of this genotype in both recipients and donors would be very helpful for adequate immunosuppressive regimen which included Tac.

Obviously, there are real expectations that genetic testing before organ transplantation can predict individual response to certain immunosuppressive drugs. This would lead to improved treatment, as well as better graft survival after renal transplantation in future.

Conclusion

The present study is the first analysis of the distribution of gene polymorphisms encoding CYP3A5, CYP3A4 and ABCB1 in patients subjected to renal transplantation in our transplantation center. A total of 84.8% of our patients were found to express both the CYP3A5*3*3 genotype, associated with diminished CYP3A5 enzymatic activity, and CYP3A4*1*1/*1*1B, associated with functional CYP3A4 enzymatic activity, while out of all the patients with diminished CYP3A5 enzymatic activity, 68.7% had diminished activity of ABCB1 transporter. However, further studies are needed which would actually show the influence of these genetic polymorphisms on Tac blood concentrations in patients after renal transplantation.

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R E F E R E N C E S

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Caregivers' attitudes, knowledge and practices of oral care at nursing homes in Serbia

Stavovi, znanje i praksa negovatelja u održavanju oralnog zdravlja korisnika domova za stara lica u Srbiji

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Abstract

Background/Aim. Within the elderly population, residents in nursing homes, there is a greather risk of caries, periodontal disease and teeth loss. Assistance of caregivers in maintaininig good oral hygiene besides improving oral health can improve of residents general health and the qulity of their lives. The aim of this study was to examine the attitudes of caregivers and knowledge about oral health, as well as the practice regarding oral care they apply at nursing homes in Serbia. **Methods.** The survey was conducted at the Gerontology Center Belgrade, consisting of four nursing homes located in the urban area. The study included 58 caregivers. They were contacted on working days, in all work shifts, during January, February and March of 2013. They were asked to fill in a self-administered questionnaire consisting of 26 closed-type questions. **Results.** The caregivers mostly considered that it was very important to take care of oral health of the residents, but 69% responded that the level of their oral health was low or very low. As the main barriers to oral hygiene maintenance, the caregivers indicated lack of time. The caregivers had more knowledge about periodontal disease than about the main cause of caries and its prevention. Formal medical education had the influence on the knowledge about oral diseases. Oral hygiene procedures carried out by the mayority of caregivers were denture cleaning and tooth brushing. **Conclusion.** The caregivers were aware of the limitations in everyday oral care of nursing homes residents in Serbia, although solving these problems requires the involvement of the entire public health service.

Key words:
old age assistance; homes for the aged; oral hygiene; comprehensive dental care; serbia.

Apstrakt

Uvod/Cilj. U grupi starijih osoba, stanovnika domova za stara lica, postoji povećani rizik od nastanka karijesa, periodontalne bolesti i gubitka zuba. Pomoć u održavanju dobre oralne higijene, pored toga, može uticati i na opšte zdravstveno stanje i kvalitet života stanovnika domova za stara lica. Cilj ovog istraživanja bio je da se ispitaju stavovi i znanje negovatelja o oralnom zdravlju, kao i praksa koju primjenjuju u domovima za stara lica u Srbiji. **Metode.** Istraživanje je sprovedeno u ustanovi Gerontološki Centar Beograd, koji se sastoji od četiri doma za stara lica, locirana u urbanom području. U istraživanju je učestvovalo 58 negovatelja, s kojima smo kontaktirali radnim danima, u svim radnim smenama, tokom januara, februara i marta 2013. godine. Oni su zamoljeni da samostalno ispune upitnik sastavljen od 26 pitanja zatvorenog tipa. **Rezultati.** Negovatelji uglavnom smatraju da je veoma važno brinuti se o oralnom zdravlju korisnika domova, ali 69% je odgovorilo da je nivo oralnog zdravlja korisnika nizak ili vrlo nizak. Za glavnu prepreku u održavanju oralne higijene korisnika domova, negovatelji su označili nedostatak vremena. Njegovatelji su imali više znanja o parodontalnim bolestima, nego o glavnom uzročniku karijesa i njegovoj prevenciji. Formalno medicinsko obrazovanje imalo je uticaj na njihovo znanje o oralnim bolestima. Čišćenje proteza i pranje zuba su najčešće primenjivani postupci negovatelja u održavanju oralne higijene korisnika domova. **Zaključak.** Negovatelji su svesni ograničenja u svakodnevnom održavanju oralnog zdravlja korisnika domova za stara lica u Srbiji, kao i da rešavanje tih problema zahteva uključivanje celokupnog javnog zdravstvenog sektora.

Ključne reči:
stare osobe, pomoć; staračkі domovi; usta, higijena; zubi, nega i lečenje; srbija.

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Introduction

An increasing number of older people and their needs for health care is a challenge for public health services, including oral health care [1]. Within the elderly population there is a greater risk of caries, periodontal disease and teeth loss, especially among functionally dependant and cognitively impaired residents at nursing homes [2]. Poor oral health of nursing home residents is mostly the result of the absence or difficult access to the professional dental care, lack of motivation, difficulties in motor skills and poor medical status of nursing homes residents, but also inadequate practice and the attitude of caregivers [3]. Oral disease in institutional facilities can affect residents' systemic health [2]. Bacterial endocarditis, pneumonia and pulmonary abscess can be the result of the anaerobic bacteria infection; the dental plaque is the source of these bacteria, especially in patients with periodontal disease [4,5]. Therefore, assistance in maintaining good oral hygiene besides improving oral health can also improve residents' general health and the quality of life [6].

Numerous studies have shown that lack of time, the absence of organizational support, fear of performing oral care, lack of cooperation and interest in oral care of the residents are the main problems of caregivers in preserving oral health care at nursing homes [7–14]. As a conclusion of those studies, the authors indicate that the improvement of caregivers' education and training programmes in this field are necessary [1]. There are no relevant data in Serbia about oral health care in nursing homes for elderly.

The assessment of the attitudes of caregivers regarding oral health is very important, considering the fact that they have direct influence on the effect of oral care of the residents [1]. It can provide insight into their priorities and thus into oral care practices at nursing homes.

The aim of this study was to examine the attitudes of caregivers and their knowledge about oral health, as well as the practice regarding oral care they apply at nursing homes in Serbia.

Methods

Study sample

The study was conducted at the Gerontology Center Belgrade, consisting of four nursing homes located in the urban area. This institution has the capacity of nearly 1,200 residents, with 113 caregivers. The study included 58 out of 113 caregivers, with the response rate of 51%. The caregivers were contacted on working days, in all work shifts, during January, February and March of 2013. The criterion for inclusion in the study was permanent employment in the Gerontology Center and voluntar participation to the study. The protocol for this study was approved by the local ethics committee (No. 36/31) at the Faculty of Dental Medicine, University of Belgrade.

Questionnaire

The survey was conducted using the self-administered questionnaire consisting of 26 closed-type questions. The questionnaire was designed to provide information on demographic characteristics of caregivers, caregivers profession and training in oral care, attitudes towards oral health care of nursing homes residents, practices applied in relation to providing oral health care, knowledge of aetiology and manifestation of oral diseases, and caregivers knowledge of denture hygiene.

Statistical analysis

The obtained data were analysed using descriptive statistics in the Statistic Software SPSS (version 11.5 for Windows, SPPS Inc., Chicago, IL, USA). Categorical data were compared using the χ^2-test and Mann-Whitney test for between-group differences. A statistical significance was determined at $p = 0.05$ for all tests.

Results

The study included 58 caregivers (5 men and 53 women), with the mean age of 43.9. Significantly more caregivers had no formal medical education (70.7%, $p < 0.05$). Most of the caregivers (84.5%) were responsible for over 20 residents, while 15.5% were responsible for 15–20 residents. The majority of the caregivers (37.9%) had over 20 years of experience in working with the elderly.

Training in assisting residents in maintaining oral hygiene passed 81% of the respondents. Training in causes, course and prevention of oral diseases passed 36.2% of the caregivers, out of whom 78% considered it useful in practice. Most caregivers had learned oral hygiene techniques from colleagues (41.4%). Also, most of them (96.6%) considered that it was very important to take care of residents' oral health. When asked to evaluate the level of residents' oral health, 69% responded as low or very low. Lack of time of the caregivers was indicated as the main barrier to oral hygiene maintenance (Figure 1).

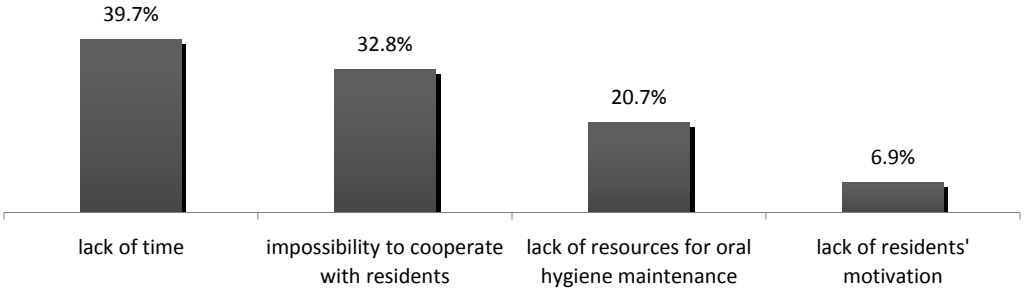

Fig. 1 – Main barriers to maintaining oral hygiene of the residents.

When it comes to oral hygiene procedures which the caregivers apply to the residents, most of them applied only denture cleaning, while significantly fewer rinsing with oral solutions (Figure 2). A total of 79.3% of the caregivers carried out oral hygiene procedures once a day, 8.6% 2–3 times a week, while 12.1% of the caregivers did not carry any oral hygiene procedure.

The knowledge of caregivers on the symptoms and prevention of caries and periodontal disease is shown in Table 1. As a possible cause of dental caries, most caregivers reported poor oral hygiene. Also, most of them answered that tooth decay could be best prevented by regular brushing and visits to the dentist. As the main cause of gum disease, they considered bacteria in dental plaque with swollen gums that easily bleed to gentle wash as the main symptom.

Comparing the responses to the question about the possible causes of tooth decay among the staff with and without formal medical education (FME), there was a statistically significant difference in the answers "Poor oral hygiene" and "Lack of calcium in the diet" (Table 2). Also, in the question about dental caries prevention, a statistically significant difference was observed in the response "Regular dental exams" (Table 2). Regarding oral hygiene procedures, there was a statistically significant difference ($p < 0.05$) in the responses "Cleaning dentures" and "None of the above", between respondents who had received oral care training and those who did not (Table 3).

Most of the respondents (81%) answered that the best way to clean dentures was the use of a toothbrush, toothpaste and soaking in a denture cleanser, and 79.3% answered that dentures should be kept in water when they were not in the mouth. In addition, most of the caregivers (77.6%) considered that dentures should not be left in the mouth at night, and 72.4% stated that broken dentures could be repaired. Most respondents (81%) agreed that prostheses should be cleaned at least once a day and washed after each meal.

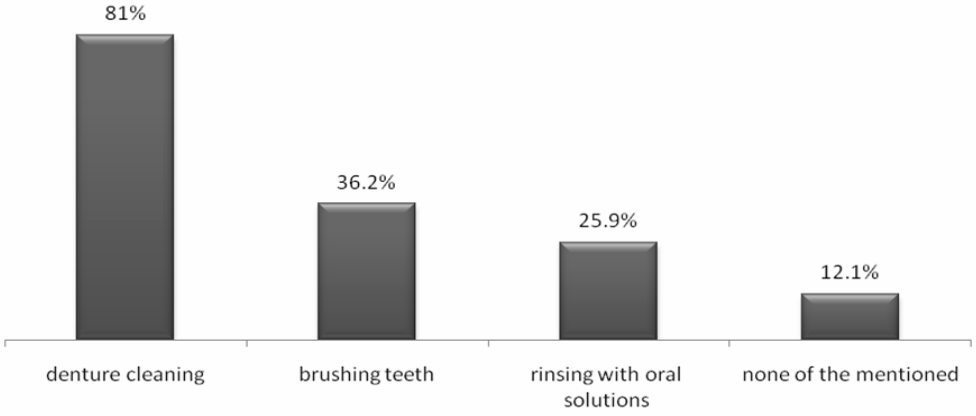

Fig. 2 – Procedures that the caregivers apply to maintain oral hygiene of the residents.

Table 1

Caregivers' knowledge of symptoms and prevention of caries and periodontal disease

Questions on oral health	Caregivers' responses, n (%)
Aetiology of dental caries	
poor oral hygiene	43 (74.1)
frequent intake of sugary food and drinks	29 (50.0)
lack of calcium in the diet	27 (46.6)
Prevention of dental caries	
regular brushing and regular dental check-ups	41 (70.7)
restricted intake of sugary food and drinks	17 (29.3)
using the oral solutions with fluoride	18 (31.0)
Signs and symptoms of gum disease	
bad breath	36 (62.1)
swollen gums which bleed easily on gentle brushing	45 (77.6)
loose teeth	35 (60.3)
the space between the teeth	4 (6.9)
sensitive teeth	14 (24.1)
holes in teeth	12 (20.7)
The main cause of gum disease	
bacteria in dental plaque	46 (79.3)
sugar in sweet food and drinks	14 (24.1)
poor nutrition	15 (29.5)

Table 2

Comparison of the responses given by the caregivers with and without formal medical education (FME)

Questions on oral health	Caregivers without FME	Caregivers with FME	χ^2-test *(p)*
	n (%)	n (%)	
Aetiology of dental caries			
poor oral hygiene	27 (65.9)	16 (94.1)	0.025
frequent intake of sugary food and drinks	22 (53.7)	7 (41.2)	0.387
lack of calcium in the diet	23 (56.1)	4 (23.5)	0.024
Prevention of dental caries			
regular brushing	29 (70.7)	12 (70.69)	0.991
restricted diet of sugary food and drinks	14 (34.1)	3 (17.6)	0.209
using the oral solutions with fluoride	13 (31.7)	5 (29.4)	0.863
regular dental check-ups	25 (61.0)	16 (94.1)	0.012
The main cause of gum disease			
sugar in sweet food and drinks	8 (19.5)	6 (35.3)	0.201
bacteria in dental plaque	33 (80.5)	13 (76.5)	0.731
poor nutrition	8 (19.5)	7 (41.2)	0.086

Table 3

Comparison of the applied procedures by the caregivers with and with no oral care training

Procedures for maintaining oral hygiene	Caregivers with oral care training, n (%)		χ^2-test *(p)*
	yes	no	
Brushing teeth	19 (40.4)	2 (18.2)	0.167
Using the oral solutions with fluoride	14 (29.8)	1 (9.1)	0.158
Dentures cleaning	41 (87.2)	6 (54.5)	0.013
None of the mentioned	3 (6.4)	4 (36.4)	0.006

Discussion

The limitation of the study derives from its descriptive nature, but the results are nonetheless very significant, given that similar studies have not been conducted in Serbia. Regarding the response rates, other studies in similar contexts showed the response rate of 25% [15] and 75% [16] of the sample. In this study, the response rate was 51%. Obtaining a higher response rate is difficult due to high staff turnover, sick leave and scheduling. Most of the caregivers who participated in this study were women, that coincided with the results of other studies [1].

Oral care training had been attended by 81% of the study participants and almost the same percentage considered it useful in everyday practice. However, what remains as a main problem is the absence of protocols and standards which would institute these courses and trainings as one of the priorities in nursing homes and an obligation for every staff member.

The study showed that almost all the staff members had a positive attitude towards the importance of oral hygiene in nursing homes, which is very encouraging, since they are dentists` partners in achieving mutual aim, namely oral health improvement for nursing homes residents. However, the fact that nearly two-thirds of caregivers consider the current state of residents' oral health as bad or really bad, reporting lack of time as the main problem that prevents them from dedicating more time to oral care, raises a huge concern. This is in contrast with the results from other studies, where the impossibility of cooperation with nursing homes residents [17] or the lack of residents' will [18] is stated as the main barrier. In addition to lack of time, a great number of residents have the same caregiver during the one work shift.

The nursing home staff had more knowledge about periodontal disease than about the main cause of caries and its prevention, is the same as in other studies [5].

The results showed that FME influenced the knowledge about oral diseases. The majority of caregivers with FME (94.1%) said that "poor oral hygiene" was a possible cause of caries, and for caregivers without FME the number was slightly lower (65.9%). In contrast to that, 23.5% of caregivers with FME responded positively to the given answer "The lack of calcium in nutrition", compared to more caregivers (56.1%) without FME who answered positively, which shows that the staff with FME was more informed about the causes of oral diseases. The fact that 94.1% of caregivers with FME stated that caries might be prevented by regular dentist check compared to 61.0% of caregivers without FME who answered the same supports this conclusion. Such results indicate that staff with FME is able to take better care of oral health of nursing home residents and that additional training in oral diseases is necessary for caregivers without medical and/or dental education.

Regarding knowledge about denture hygiene, caregivers showed a high level of information, mostly gained through their practical experience.

Oral hygiene procedures carried out by most caregivers are dentures cleaning (81%) and tooth brushing (36.2%). The concerning fact is that, in contrast to other studies [1], our study showed that there is a number of staff (12.1%) who did not apply any procedure in order to maintain oral hygiene of nursing homes residents. However, this information does not have to be strictly negative, since it may be the result of the presence of functionally independent residents who do not need help in maintaining oral hygiene.

Several studies highlight oral health care as an important part of elderly health care [19, 20]. Furthermore, they show that nursing homes are responsible for the help in residents everyday oral hygiene maintenance, but when countries in economic transition are concerned, there are very few data considering this topic. In developing countries, caregivers lack of education is common, and this particularly refers to those working in geriatric centers [21]. A study [22] shows how much dental teams contribute to supporting and encouraging a nursing home staff in maintaining continuous oral health care. Also, previous studies have shown a significant improvement in staff's attitudes and approaches towards oral health care after a short educational program for nursing attendants [23–26]. More quality training may provide better oral care, but there are few studies describing these effects [27]. Some studies [28, 29] show that skills of caregivers at nursing homes in providing oral care are improved several months after an oral care training course, but they have not succeeded in showing the influence on the residents' oral health.

Conclusion

It is important that nursing attendants are aware of the main barriers and problems in everyday oral care of nursing homes residents in Serbia, although solving these problems is complex and requires the involvement of the entire public health service. The lack of time, as the main obstacle in maintaining residents' oral health, may be solved with better staff organization, as well as with providing more caregivers in order to relieve the current staff from numerous obligations. Dental profession has to emphasize the importance of oral hygiene and of educating the nursing home staff in order to improve oral health care at nursing homes.

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CASE REPORTS

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Urgent carotid stenting before cardiac surgery in a young male patient with acute ischemic stroke caused by aortic and carotid dissection

Hitno ugrađivanje stenta u karotidu pre kardiohirurške intervencije kod mladog muškarca sa akutnim ishemijskim moždanim udarom izazvanim aortnom i karotidnom disekcijom

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Abstract

Introduction. Acute aortic dissection (AD) is the most common life-threatening disorder affecting the aorta. Neurological symptoms are present in 17–40% of cases. The management of these patients is controversial. **Case report.** We presented a 37-year-old man admitted for complaining of left-sided weakness. Symptoms appeared two hours before admission. The patient had no headache, neither thoracic pain. Neurological examination showed mild confusion, left-sided hemiplegia, National Institutes of Health Stroke Scale (NIHSS) score was 10. Ischemic stroke was suspected, brain multislice computed tomography (MSCT) and angiography were performed and right intrapetrous internal carotid artery dissection noted. Subsequent color Doppler ultrasound of the carotid arteries showed dissection of the right common carotid artery (CCA). The patient underwent thoracic and abdominal MSCT aortography which showed ascending aortic dissection from the aortic root, propagating in the brachiocephalic artery and the right CCA. Digital subtraction angiography was performed subsequently and two stents were successfully implanted in the brachiocephalic artery and the right CCA prior to cardiac surgery, only 6 hours after admission. The ascending aorta was reconstructed with graft interposition and the aortic valve resuspended. The patient was hemodynamically stable and with no neurologic deficit after surgery. Unfortinately, at the operative day 6, mediastinitis developed and after intensive treatment the patients died 35 days after admission. **Conclusion.** In young patients with suspected stroke and oscillatory neurological impairment urgent MSCT angiography of the brain and neck and/or Doppler sonography of the carotid and vertebral artery are mandatory to exclude carotid and aortic dissection. The prompt diagnosis permits urgent carotid stenting and cardiosurgery. To the best of our knowledge, this is the first published case of immediate carotid stenting in acute ischemic stroke after the diagnosis of carotid and aortic dissection and prior to cardiac surgery.

Key words:
aorta; carotid artery, internal, dissection; stroke; cardiovascular surgical procedures; stents; young adults; treatment outcome.

Apstrakt

Uvod. Disekcija aorte je najčešće i po život opasno oboljenje aorte. Neurološki simptomi se javljaju kod 17–40% bolesnika. Lečenje ovih bolesnika je predmet kontroverzi. **Prikaz bolesnika.** Prikazali smo bolesnika starog 37 godina, koji je primljen u centar hitne pomoći zbog slabosti levostranih ekstremiteta. Simptomi su se javili dva sata pred prijem. Bolesnik nije imao glavobolju, ni bolove u grudima. Somatski pregled bio je bez patološkog nalaza. U neurološkom pregledu objektivizirana je laka konfuznost, plegija levostranih ekstremiteta, centralni facijalis levo, ekstenzioni plantarni odgovor levo *National Institutes of Health Stroke Scale* (NIHSS) skor bio je 10. Posumnjano je na ishemijski infarkt mozga, te je odmah urađeno snimanje glave multislajsnim skenerom (MSCT) sa angiografijom, na kome je uočena suspektna disekcija intrapetroznog dela desne unutrašnje karotidne arterije (ACI). Nakon toga urađen je dopler krvnih sudova vrata na kome je uočena disekcija desne zajedničke karotidne arterije. Urađena ekstrakranijalna MSCT angiografija ukazala je na disekciju luka aorte koja se širila ka brahiocefaličnom stablu i desnoj zajedničkoj karotidnoj arteriji. Odmah je urađena digitalna suptrakciona angiografija i u istom aktu postavljenja su dva stenta u brahiocefalično stablo i desnu zajedničku karotidnu arteriju. Šest sati nakon prijema, zbog disekcije ascedentne aorte, bolesnik je podvrgnut operaciji, kada je rekonstruisana ascendentna aorta i

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urađena resuspenzija aortnog zaliska. Šestog postoperativnog dana razvio se medijastinitis. Lečenje je pokušano visokim dozama antibiotika, međutim 35. postoperativnog dana bolesnik je preminuo. **Zaključak.** Kod mlađih bolesnika sa sumnjom na infarkt mozga i promenljivim neurološkim deficitom hitna MSCT angiografija glave i vrata ili dopler karotidnih i vertebralnih arterija su obavezni u cilju isključivanja aortne i karotidne disekcije. Brza dijagnoza omogućava hitnu ugradnju stenta u karotidu i kardiohirurgiju. Prema nama dostupnim podacima iz literature, ovo je prvi objavljen prikaz hitne ugradnje stenta u karotidu kod akutnog infarkta mozga nakon dijagnoze karotidne i aortne disekcije, a neposredno pre kardiohirurške intervencije.

Ključne reči:
aorta; a. carotis interna, disekcija; mozak, veliki, infarkt; hirurgija, kardiovaskularna, procedure; stentovi; mlade osobe; lečenje, ishod.

Introduction

Acute aortic dissection (AD) is the most common life-threatening disorder affecting the aorta. The incidence of AD ranges between 5 and 30 cases *per* million people *per* year [1]. The outcome of aortic dissection is frequently fatal, and many patients may die before presentation to the hospital or before diagnosis.

More than one third of all patients with aortic dissection demonstrate signs and symptoms secondary to organ system involvement [2]. Neurological symptoms at onset of aortic dissection are not rare, they are present in 17–40% of cases, (ischemic stroke, spinal cord ischemia, ischemic neuropathy, hypoxic encephalopathy, syncope) [2], but aortic dissection remains often unrecognized as underlying cause and life threatening condition. Especially in pain-free dissections with predominant neurological symptoms diagnosis of aortic dissection can be difficult and delayed.

We presented a young patient with acute onset of left-sided hemiplegia as a sole clinical manifestation of acute aortic and carotid dissection. The patient was promptly diagnosed and underwent urgent percutaneous carotid stenting in order to stabilize neurologic deficit followed by immediate cardiac surgery repair.

We would like to stress two issues. The first one is that all young stroke patients should have MSCT angiography of the brain and neck arteries. The second one is that prompt stenting of the carotid artery dissection in acute ischemic stroke enables surgery of aortic dissection.

Case report

We presented a 37-year-old man admitted to the Emergency Department of Military Medical Academy (MMA) complaining of left-sided weakness. Symptoms appeared two hours before admission, after the patient woke up in the morning. He had no other complaints, nor headache, neither thoracic pain. Risk factors in the presented patient were obesity, history of hypertension and smoking. On general examination the patient was conscious, afebrile, blood pressure was 150/90 mmHg, pulse 86/min, auscultation revealed normal heart and lung sounds, ECG showed no abnormal findings. Neurological examination showed mild confusion, left-sided hemiplegia and central facial palsy on the left side with left extensor plantar response, National Institutes of Health Stroke Scale score was 10, Modified Rankin Scale (mRS) was 4. Ischemic stroke was suspected and the patient was considered for intravenous thrombolysis though exact time of symptom onset was uncertain. Brain multislice computed tomography (MSCT) and brain MSCT angiography were performed within 45 min of admission showing no brain lesions, however right intrapetrous internal carotid artery (ICA) dissection was suspected (Figure 1).

Subsequent color Doppler ultrasound of the carotid arteries showed dissection of the right common carotid artery (CCA) (Figure 2).

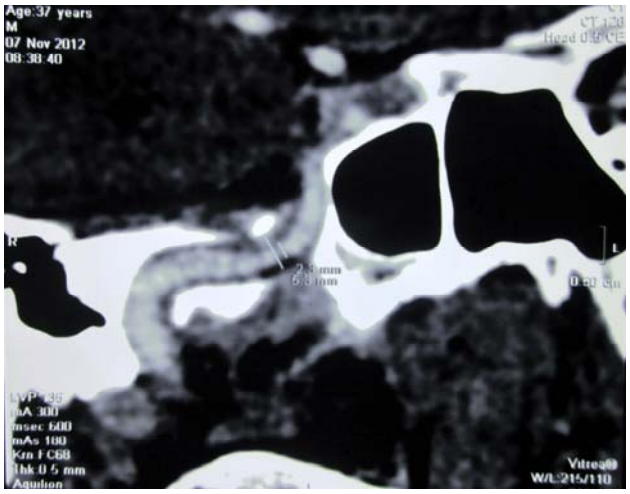

Fig. 1 – Multislice computed tomography (MSCT) angiography of the brain. A petrous segment of the right internal carotid artery narrowed approximately 60%.

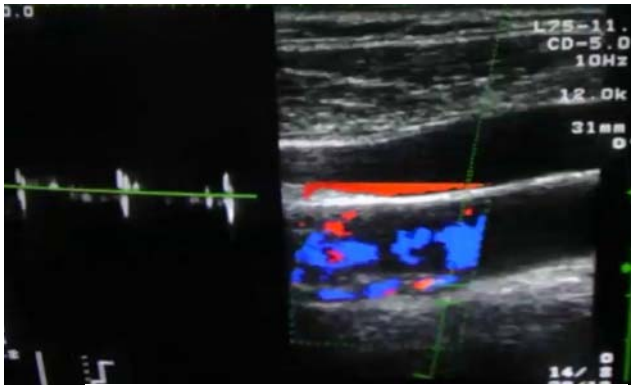

Fig. 2 – Doppler ultrasound of carotid arteries. Biphasic flow of the right common carotid artery.

Meanwhile, within 3 hours from the symptom onset, there was an almost complete neurological deficit reduction, with only mild left-arm weakness persisting, NIHSS score 2. The patient underwent abdominal, thoracic and extracranial

MSCT angiography which showed ascending aortic dissection Stanford type A, propagating in the brachiocephalic artery (BCA) and the right CCA (Figure 3). Following MSCT, digital subtraction angiography (DSA) was performed and two self-expanding stents (8 × 60 mm Protege GPS self expanding peripheral stent, manufactured by EV3) inserted in the brachiocephalic artery and the right CCA with complete closure of dissection with establishing normal anterograde flow (Figure 4). Due to dissection of the ascending aorta the patient underwent emergency cardiosurgery, 8 hours after symptom onset and 6 hours after admission to the hospital. Resection of the ascending aorta and reconstruction with the arteficial blood vessel by the use of graft-interpositum was performed as well as resuspension of the aortic valve. Surgery was performed in deep hypothermic circulatory arrest with antegrad cerebral perfussion, with flow of 500 ml/min, for 42 minutes. A pathohystologic sample of aortic tissue showed possible cystic medial necrosis in the aortic tissue. After the surgery, the patient was admitted to the postoperative intensive care unit. The aortic valve was competent, with aortic regurgitation 1+, compared with 3+ before the surgery. On the postoperative day 6 the patient developed mediastinitis, treated conservatively with antibiotics and general supportive measures. The patient died 35 days later due to complications of mediastinitis.

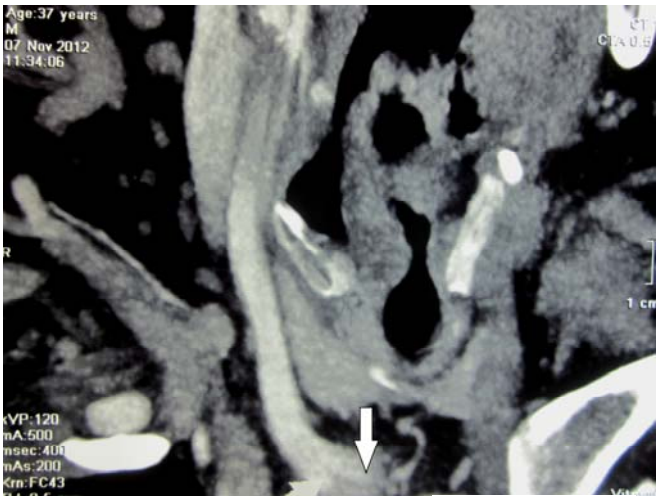

Fig. 3 – Multislice computed tomography (MSCT) angiography of carotid arteries shows dissection of the common carotid artery with subtotal prebulbar stenosis (arrows) and right internal carotid artery non-significant calcified stenosis.

Fig. 4 – Digital subtraction angiography. Stanford type aortic dissection. Stents in brachiocephalic artery. Arrows show the false lumen of the aorta.

Discussion

Our patient was presented with left-sided hemiplegia as the first and only sign of aortic and right-sided brachiocephalic artery dissection. The diagnosis was made and confirmed within the first two hours of admission, subsequent interventional radiology with brachiocephalic stenting of dissection made within three hours of addmission and cardiothoracic surgery six hours after admitance to the hospital, i.e. three hours after the diagnosis was confirmed. According to the present guidelines, emergency imaging of the brain is recommended before initiating any specific therapy to treat acute ischemic stroke (Level A, Class 1) [3]. A non-invasive intracranial vascular study is strongly recommended during the initial imaging evaluation of acute stroke patients if intravenous thrombolysis is contemplated for management but should not delay if thrombolysis indicated [3]. However, considering higher percentage of dissection as the cause of ischemic stroke in younger patients, brain and neck MSCT angiography and Doppler sonography of carotid and vertebral arteries (if MSCT is not available) should be part of mandatory diagnostic procedures in this patient population. If MSCT brain angiography had not been performed in our patient, carotid dissection would not have been suspected and further diagnostic would not have been performed, leaving aortic dissection unrecognized. Thrombolytic treatment in acute ischemic stroke related to internal carotid artery dissection has been reported to be safe and effective [4]. However, aortic dissection is the absolute contraindication for thrombolysis. So, in case of confirmed carotid stenosis aortic dissection has to be excluded in order to proceed with thrombolysis.

Regarding stenting of the brachiocephalic and carotid artery combined with aortic dissection surgery, there are no clear guidelines [5]. Sporadic case reports are published, with stenting after, or during the surgery of aortic arch (hybrid procedures) [6–8], by performing temporary femoral-carotid artery bypass [9], or by successful repair of the brachiocephalic artery followed by reconstruction of the ascending aorta with the patient in profound hypothermia [10]. To the best of our knowledge, there has been no published report on carotid stenting in acute ischemic stroke prior cardiosurgery. The official guidelines confirm controversy over whether surgery should be performed in patients with AD type A presenting with neurological deficits [5]. Although commonly associated with a poor postoperative prognosis, recovery has been reported when rapid brain reperfusion is achieved [5], as it was in the presented case by brachiocephalic and carotid stenting. This is the first case that such hybrid procedure, carotid stenting/cardiac surgery, has been attempted and completed in our hospital.

Surgical mortality for acute AD type A reported in different experiences from single centers or surgeons varies from 7% to 30% [11]. The immediate mortality rate in aortic dissection is as high as 1% *per* hour over the first 48 hours, making early diagnosis and treatment critical for survival [12, 13]. In the International Registry of Acute Aortic Dissection, data related to symptomatic aortic dissection, median time from arrival to the emergency department and diagnosis was 4.3 hours, and from diagnosis to surgery also 4.3 hours [14]. Delays in recognition are mainly because of nonexistence of characteristic signs and symptoms, and delays are prolonged in non-tertiary hospitals due to lack of equipment and skilled stuff. Our patient experienced neurological impairment due to non-symptomatic aortic dissection, making the diagnosis even more challenging. Nevertheless, the diagnosis was made within the 3 hours of admission, carotid stenting within 4 hours and surgery within 6 hours of admission.

Most affected patients complain of sudden, severe chest pain. Whereas most patients without neurological symptoms (94%) experience initial pain, only two-thirds of patients with neurological symptoms at the onset of dissection report a history of pain [2]. Our patient complained of no thoracic pain, nor headache.

The frequency of neurological involvement varies from 17% to 40% [2]. Neurological symptoms can be classified into different groups: ischemic stroke, spinal cord ischemia, ischemic neuropathy, and hypoxic encephalopathy. However, symtoms of acute ischemic stroke are the most common initial neurological finding. Strokes tend to be most frequently hemispheric and predominantly in the right hemisphere. Involvement of the major branches of the aortic arch varies from 20% to 43% [2]. Our patient was presented with signs of acute ischemic stroke of the right hemisphere and dissection of the right CCA.

Symptoms of the carotid artery dissection are grouped into triad of unilateral head, facial, or neck pain with accompanying partial Horner syndrome [4]. However, less than one-third of patients are presented with this classic triad [4]. Headache presents in 44–69% of patients and partial Horner syndrome may be found in up to 50% of patients [4]. In addition, approximately 5% of carotid artery dissections are asymptomatic [4]. Our patient showed neither of these signs nor did he complained of any pain in neck or head.

Carotid artery dissection is the underlying cause of approximately 2.5% of strokes in all patients [4]. Nevertheless, it accounts for up to 20% of strokes in patients younger than 45 years [4], as was our patient.

As aforementioned, in younger patients brain CT angiography, extracranial CT angiography or ultrasound of carotid arteries should be performed, although there are no official recommendations for the last two. This is especially important when such diagnostic procedures are available in medical facilities and there is no time loss as was the case with the presented patient.

Early detection of acute ischemic stroke secondary to painless aortic dissection is challenging itself, not only due to narrow 4.5 hours' time window for thrombolytic therapy, but due to possible interventional radiology and cardiosurgery procedures.

After confirming dissection, our patient underwent brachiocephalic and carotid stenting, before surgical reparation. Surgery itself is a great risk, emphasized with duration of surgery, urgency of procedure and obesity of patients. These are also risk factors for mediastinitis. Unfortunately, mediastinitis developed in the presented patient and he died 35 days after the surgery. Apart from the prompt diagnosis by the neurologist, experienced interventional radiologist capable of instant carotid stenting, availability of immediate cardiac surgery, postoperative intensive care is likewise crucial for the survival.

Conclusion

In young patients with suspected stroke and oscillatory neurological impairment MSCT angiography of the brain and neck or Doppler sonography of the carotid and vertebral arteries are mandatory to exclude carotid and aortic dissection especially if thrombolytic treatment is contemplated. The prompt diagnosis permits urgent carotid stenting and cardiosurgery. To the best of our knowledge, this is the first published case of immediate carotid stenting after the diagnosis of carotid and aortic dissection and prior to cardiosurgery.

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CASE REPORT

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Gigantic spermatocytic seminoma – A rare tumor of germ cell origin

Veliki spermatocitni seminom – redak tumor sa poreklom iz ćelija zametka

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Abstract

Introduction. Spermatocytic seminoma represents a rare hystologic type of malignant testicular germ cell tumor with slow course and low malignant potential. **Case report.** We presented a 69-year-old patient with atypical clinical presentation of spermatocytic seminoma initially diagnosed as gigantic hydrocoellae which compromised walking. After long term evolution clincal picture presented with signs and symptoms of acute scrotum. Preoperative echosonography was performed and the diagnosis of testicular infiltrative tumor was established. After that left scrotal orchiectomy was performed. Patohistological examination revealed spermatocytic seminoma. **Conclusion.** Inspite good prognosis there is a low probability of development of high grade malignancy sinchronous sarcoma within the testis with a high potential for lymphogenic and hematogenic dissemination. Individual approach is necessary in accordance with the pathohistological diagnosis.

Key words:
seminoma; diagnosis; diagnosis, differential; urogenital surgical procedures; treatment outcome.

Apstrakt

Uvod. Spermatocitni seminom predstavlja retku varijantu malignog tumora testisa benevolentnog kliničkog toka i povoljne prognoze. **Prikaz bolesnika.** U radu je prikazan bolesnik, starosti 69 godina, sa atipičnom prezentacijom spermatocitnog seminoma koji je incijalno dijagnostikovan kao gigantska bilateralna hidrocela koja je kompromitovala hodanje. Nakon dugogodišnje evolucije bolest se ispoljila sa simptomima i znacima akutnog skrotuma. Preoperatvnom ehosonografijom postavljena je dijagnoza infiltrativnog tumora levog testisa, a potom je učinjena leva skrotalna orhiektomija. Patohistološkim pregledom preparata postavljena je dijagnoza spermatocitnog seminoma testisa. **Zaključak.** Uprkos povoljnoj prognozi za ovaj histološki tip, postoji potencijalna evolucija u pravcu razvoja sarkomske komponenete u tumoru sa visokim malignim potencijalom i mogućnošću nastanka sistemskih visceralnih i limfonodalnih metastaza. Potreban je individualni pristup svakom bolesniku u skladu sa definitivnom patohistološkom dijagnozom.

Ključne reči:
seminom; dijagnoza; dijagnoza, diferencijalna; hirurgija, urogenitalna, procedure; lečenje, ishod.

Introduction

Testicular malignant tumors represent about 1% of all malignant tumors with peak incidence in population of younger men, age interval 15–34 years. Also they are dominant cause of death caused by malignant tumors in this population. More than 90% of these tumors arise from germ cells and they are classified into two subgruops: seminomatous and non-seminomatous tumors (yolk sac carcinoma, embrional carcinoma, choriocarcinoma and teratoma). Less than 6% arises from testicular stromal cells (Sertoli and Leydig cells). The smallest group of testicular tumors have mesenchymal origin (primary sarcoma and lymphoma of the testis) [1–3]. In the era before chemotherapy, which is recognized nowdays as powerfull adjuvant treatment option testicular cancer was highly incurable neoplasm with a 5-year survival lower than 5%. With introduction of platinum based chemoregimens 5-year survival increased up to 99% for the first stage (tumor confined on the testis), 96% for the stage two (locoregional spread to retroperitoneal lymph nodes), and 73% for metastatic disease (visceral metastasis in liver, lung and brain) [4, 5].

Precancerous lesion present in the testis which preceds the devolpment of cancer is called intratubular germ cell neoplasia, and the most common types of germ cell cancer which arises from it are seminoma and embrional carcinoma. Also, it is very common that testicular cancer has two or mo-

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re various histologic types which is important for the choice of adjuvant treatment option.

Unilateral, fast, painless testicular enlargement is highly suspicious for the presence of testicular tumor and is followed with elevation of blood serum tumor markers: lactat-dehidrogenase, beta chorionic gonadotropine and alfa fetoproteine.

Spermatocytic seminoma is a rare histologic type of testicular cancer which arises from mature spermatogonia in the absence of intratubular germ cell neoplasia. Peak incidence is in the population of elfarly men, age 50–60 years, and rarely is present in men aged below 30 years. It is a low-grade malignancy which tends to grow slowly and has a low potential for systemic spread. Macroscopically, it is a nodular tumor with fields of necrosis and haemorrhage. Microscopically, three populations of cells are present with the absence of fibrous septa and lymphocytic infiltration which is common in the classical seminoma. The overall prognosis is excellent [1].

Case report

We presented a 69-year-old patient with gigantic spermatocytic seminoma manifested with signs and symptoms of acute scrotoma. Many years before the diagnosis of bilateral hydrocoelae was made. Before admittance to the hospital the only present symptom was the difficulty to walk due to the scrotal enlargement. Due to the presence of the symptoms consistent with acute scrotume (testicular swelling conjoined with severe pain followed with vegetative sypmtoms), the patient was admitted and the preoperative diagnostic was made. Laboratory analysis was within the referent range except lactate dehydrogenase level and erythrocyte sedimentation rate. Chest x-ray did not reveal any abnormalities and testicular echosonography reveled nodular mass in the left testicle. Free fluid between testicular sheats was absent (Figure 1). The patient undergone urgent left scrotal orchiectomy.

Pathohystological examination of the specimen was performed with standard hematoxylin eosin (HE) staining which revealed spermatocytic seminoma with the presence of lymphovascular invasion in stage T2N0M0. Adjuvant chemotherapy was not necessary in this case (Figures 2 and 3).

After one year of follow-up the patient was alive and well. There was no elevation of tumor markers, chest x ray and computed tomography (CT) scan of abdomen and pelvis did not reveal any signs of tumor locoregional and systemic spread.

Fig. 1 – Preoperative finding.

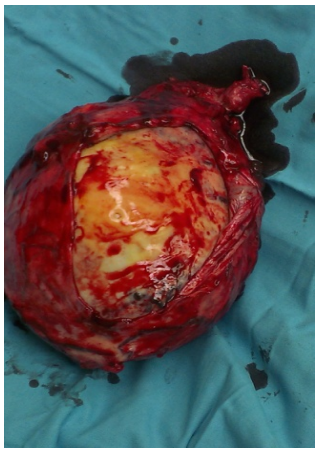

Fig. 2 – Left testis removed.

Fig. 3 – Scrotum after left unilateral orchiectomy.

Discussion

In the presented case there was an unusual manifestation of gigantic spermatocytic seminoma as acute scrotum, falsely diagnosed years ago as bilateral hydrocoelae. The true diagnosis was established on surgery which discovered spermatocytic seminoma in the low stage. Our data are consistent with literature facts regarding age, slow growth, low grade, and low metastatic potential. The accent must be made on the establishment of the correct preoperative diagnosis with available diagnostic tools.

However, although this type of tumor is rare and has the excellent prognosis, its hystogenesis and biology are not fully understood. The available data in the literature suggests that long duration of illness can lead to the development of synchronous sarcoma which has high malignant potential for systemic dissemnination [6–8].

In a case report presenting the 12th case of spermatocytic seminoma ever evolution of tumor lasted for 12 months. Development of synchronous sarcoma led to accelerated growth phase. Hystology examination revealed undiferentiated sarcoma [9].

A common sarcoma type that develops from this type of tumor is rhabdomyosarcoma [9, 10].

In another case report a patient was diagnosed with spermatocytic seminoma with synchronous rhabdomyosarcoma. Extensive metastatic disease was also present with retroperitoneal metastasis and visceral metastasis to the lungs and liver [11, 12].

Conclusion

Although spermatocytic seminoma, is a rare type of the tumor with low malignant potential, the presence of synchronous sarcoma worsens the overall prognosis. Individual approach to each patient is required in order to identify systemic spread with more frequent follow-up. Patients with the presence of synchronous sarcoma should undergo chemotherapy in order to prevent locoregional or systemic spread.

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CASE REPORT

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Orthodontic-surgical treatment of four impacted canines in an adult patient: A case report

Ortodontsko-hirurško lečenje četiri impaktirana očnjaka kod odraslog pacijenta

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Abstract

Introduction. Full impaction of canines, in both jaws, is a rare phenomenon. It is usually coupled with the persistence of deciduous canines, or any other irregularity in the dental arch. **Case report.** Panoramic radiograph of a 24-year-old female patient showed bilateral canine impaction in both jaws. Due to vestibular, apical and medial position of canines in the upper jaw, the surgical approach implied the apically positioned flap technique. The position of impacted mandibular canines was vertical with more coronal position relative to the upper canines, thus requiring a closed eruption technique. **Conclusion.** Inadequate position of impacted canines in the bone fully justifies the use of orthodontic-surgical treatment.

Key words:
tooth, impacted; radiography, dental, digital; oral surgical procedures; orthodontics; treatment outcome.

Apstrakt

Uvod. Impakcija očnjaka u gornjoj i donjoj vilici zajedno, retka je pojava. Najčešće se javlja dok su još prisutni mlečni očnjaci, kao i zbog drugih nepravilnosti zubnoga niza. **Prikaz bolesnika.** Panoramski snimak 24-godišnje pacijentkinje pokazao je obostrano impaktirane očnjake u obe vilice. Hirurški pristup oslobađanju zuba sproveden je sa apikalno pozicioniranim flapom zbog vestibularnog, apikalnog i mezijalnog položaja očnjaka u gornjoj vilici. Položaj donjih impaktiranih očnjaka bio je vertikalan sa koronalnijim položajem u odnosu na gornje očnjake, pa su hirurški tretirani metodom zatvorene erupcije. **Zaključak.** Neadekvatan položaj impaktiranih očnjaka u kosti u potpunosti opravdava primenu ortodontsko-hirurške terapije.

Ključne reči:
zub, impakcija; radiografija, stomatološka, digitalna; hirurgija, oralna, procedure; ortodoncija; lečenje, ishod.

Introduction

Tooth impaction is a very common and well-known anomaly. Although any tooth may be impacted, the most commonly impacted teeth are third molars, followed by canines, upper premolars, second lower premolars or upper incisors [1, 2]. The prevalence of impacted canines in the upper or lower jaw ranges from 0.008% up to 8.8%, whereas the upper canine impaction alone ranges from 0.8% to 2.8% [1–5]. Impacted canines are more common among females [1–6].

The prevalence of impacted canines in the upper jaw is 10 to 20 times higher compared to the lower jaw canine impaction [4]. Impacted canines can be positioned buccolabially, orally or along the crest of the alveolar ridge. The incidence of palatally impacted canines ranges from 0.27% to 2.4%, being more frequent than labially impacted canines [5]. The incidence of impacted mandibular canines ranges from 0.10% to 0.31% [2–7]. Chu et al. [6] reported 5 out of 7,486 assessed patients, i.e. 0.07% diagnosed with a mandibular canine impaction. Their position is more commonly vestibular and rarely lingual [7]. Relative to the alveolar ridge of the mandible, they may have vertical, angular or horizontal positioning.

Impaction of all four canines is a rare phenomenon, and based on the available literature, there seem to be no precise data concerning the prevalence of the respective anomaly.

The causes leading to upper canine impaction may imply: atypical position of the tooth germ prior to the formation of enamel, a long duration of eruption process, bone density, atypical position and shape of the adjacent teeth, lack of space in the dental arch or injury. Canine impaction is often accompanied by the persistence of deciduous teeth, dental ankylosis and cysts. The question whether they are the cause or the consequence of impaction, is still rather vague [8]. The main reasons for mandibular canine impaction imply: lack of space,

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supernumerary teeth, premature loss of deciduous teeth, persistence of deciduous canines, crown oversize, genetic factors, endocrine imbalance, tumors, cysts and trauma [9].

The treatment of impacted teeth may imply extraction, followed by an implant-supported or a prosthetic replacement procedure. If their position in bone allows an orthodontic-surgical treatment, the procedure consisting in surgical release and orthodontic traction of an impacted tooth to the dental arch is the method of choice. Treatment duration, surgical approach, orthodontic technique and potential problems likely to occur in the course of treatment, mainly depend on the position of the impacted tooth.

Case report

A 24 year-old female patient came to the Department of Orthodontics for orthodontic evaluation for aesthetic reasons. Clinical examination determined a protrusion of upper incisors with diastemas, severe overjet and deep overbite, with the teeth striking gingival groove. The upper jaw on the left side lacked a permanent canine, while the right side showed a persistent deciduous canine. The lower jaw assessment demonstrated persistent deciduous canines. The deciduous canines were conspicuously small and short, revealing marked attrition of the occlusal surface. The right side revealed excessive spacing, created due to the extraction of the first permanent molar. Gnatometric analysis of the study models confirmed the Class I malocclusion (Figure 1) with the protrusion of upper incisors, severe overjet (8 mm) and increased overbite (6 mm).

Fig. 1 – Pretreatment study models show protrusion of upper incisors with diastema, missing of the right maxillary permanent canine, and severe overjet and overbite.

Panoramic radiograph demonstrated bilateral impaction of permanent canines in both jaws. The analysis of the orthopantomogram showed an increased angle between the upper canines and the midline and the upper canines and the lateral incisors, which indicated their mesial inclination (Figure 2). The position of the impacted lower canines was nearly vertical.

After a detailed analysis of the study model and the radiographic imaging, we decided to apply orthodontic-surgical treatment. The results of the index on the severity of the treatment, showed a heavier treatment of the impacted upper canines and moderately heavy treatment of the impacted mandibular canines.

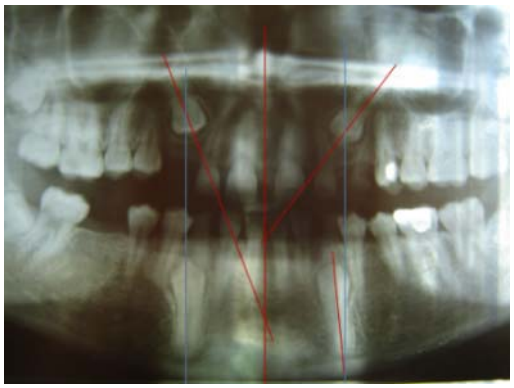

Fig. 2 – Orthopantogram analysis demonstrates vertical position of the impacted canines relative to the mucogingival line and to lateral incisors.

The aim of the pre-surgical orthodontic treatment was to ensure sufficient space for the correct positioning and alignment of permanent canines. During this phase of the treatment, other orthodontic corrections were also conducted: protrusion of upper incisors, diastema closure and overbite correction. After three months, surgical release of impacted upper canine followed. The key criteria guiding our selection of the case-specific surgical method were as follows: vertical position of the tooth in relation to the mucogingival junction, and mesiodistal position of the impacted canine crown.

The radiograph-based clinical examination identified the vestibular and apical position of the upper canines. Increased angles between the impacted teeth and lateral incisors indicated the potential risk of root resorption of the lateral incisors due to the orthodontic canine traction (Table 1). For this reason, the applied method implied surgical procedure involving the apically positioned flap technique.

Table 1

Radiological analysis of impacted canines (KPG index)

Parameters	Maxillary canines		Mandibular canines	
	right	left	right	left
Width of permanent canine (mm)	7,5	7,5	6,7	6,5
Width of the dental follicle (mm)	8,6	8,4	7,4	7,1
Grade of root development	2/3 of root	completely	completely	completely
Angle canine/midline (^{0})	16	37	0	6
Angle canine/lateral incisor (^{0})	24	40	8	1
Distance canine /occlusal plane (mm)	10	8	7,5	7
Deciduous canine	absent	non resorption	resorption without contact maxillary canine	resorption with contact maxillary canine
Evaluation of orthodontic treatments	23**	25**	7*	9*

(*) Easy to moderate orthodontic treatment (0 to 14); (**) difficult orthodontic treatment (15 to 30); KPG index – three-dimensional classification system.

Seven days after surgical release of the teeth, an elastic chain was used to attach the impacted teeth to the 0.016 × 0.022 mm steel arch wire, followed by the routing and traction of the impacted teeth (Figure 3).

The position of impacted mandibular canines did not impose any restrictions on the selection of the surgical treatment method. Given that the upper jaw was undergoing the surgical procedure involving the apically positioned flap technique, for the sake of an easier postoperative recovery and patient comfort, a surgically closed eruption procedure was conducted. Following full flap elevation and exposure of the crown, the brackets were bonded on the exposed vestibular surfaces of the impacted teeth using wire ligature. Upon completion of the flap suture, the ligature wire descending from the bonded brackets through the flap, being ligated to the existing lower 0.016 × 0.022 mm steel arch wire (Figure 4)

Traction of the canines on the left and the right side and bringing them into occlusion, was achieved by means of the postsurgical fixed orthodontic treatment. Along with the impacted canine traction procedure, the ongoing malocclusion corrections such as the protrusion of the upper incisors and the deep bite proceeded further on (Figure 5).

Since the patient was satisfied with the therapeutic and aesthetic results, upon her personal request, the treatment was discontinued after 24 months (Figures 6 a–c). A set of retainers was attached to both jaws and 18 months thereafter, the result of the treatment was quite satisfactory (Figure 7).

Discussion

The key factors disrupting the proper development and eruption of canines can lead to serious consequences in both functional and aesthetic aspect. Impacted canines may pose a risk likely to cause the occurrence of follicular cysts and infections that may threaten the lateral incisor vitality and cause their root resorption [8, 9]. If, however, the impacted canines do not cause any real problems, they often remain inside the jaw undiagnosed.

Fig. 3 – Orthodontic traction of the canine into the dental arch after surgical treatment by apically positioned flap technique.

Fig. 4 – Orthodontic-surgical treatment of impacted mandibular canines is illustrated by surgical exposure and alignment of impacted canines by the closed-eruption technique, orthodontic traction, exposure and alignment of the canines into the dental arch.

Fig. 5 – Final alignment of the canines into the dental arch.

Fig. 6 – Final results after orthodontic-surgical treatment of impacted canines: a) and b) intraoral views; c) gingival scaring of the upper right canine.

Fig. 7 – Treated canines 18 months after orthodontic-surgical treatment.

Dealing with impacted canines falls within the competence of oral surgeons, orthodontists and prosthodontists. Impaction of all four canines and the ability to achieve proper alignment into the dental arch is a challenge for any therapist. The best solution for the patient's teeth is to be naturally settled into the jaw. However, the patient's age and the position of the teeth largely determine the type of treatment [10]. Tooth extraction and implant restoration is the method recommended for adult patients [11]. However, the favorable position of impacted canines, should trigger any dentist to attempt surgical-orthodontic treatment regardless the patient's age. In this particular case, the impacted upper and lower canines were diagnosed at the age of 24. Up to that moment, the patient had not been aware, nor had she ever been warned about the presence of impacted canines. The full impaction of all four canines was coupled with the persistence of deciduous canines.

Vestibular position and mesial inclination of the impacted canine in the upper jaw was the reason for opting for the apically positioned flap method applied [10, 12]. This method enabled monitoring of the distal tooth movement. However, the labially impacted maxillary anterior teeth, treated by apically positioned flap technique, might exhibit certain aesthetic drawbacks compared to the closed eruption method [12]. Gingival scarring on mesial side of the upper right canine possibly occurred as a consequence of the surgery (Figure 6 c). However, 18 month of a retention period following the orthodontic treatment, the problem seemed to be spontaneously consolidated. An increase in clinical crown length was also determined in both canines. This may be the result of positioning of the flap during surgery [13, 14].

Looking at the size of the clinical crown of the lower canines treated by closed eruption technique, it was also observed that the clinical crown extended considerably, which did not fall within the scope of the expected results of this method [15]. No trace of gingival scarring was observed as an advantage of this method. The closed eruption method seemed to imitate the natural tooth eruption.

An index based on the position of impacted canines can predict the level of severity and duration of the treatment, indicated to the potential severe treatment of upper and a moderately severe treatment of impacted mandibular canines [16]. Therefore, the traction treatment of impacted canine was initiated in the upper jaw first. This was also confirmed based on the duration of postoperative orthodontic treatment of impacted canines in the upper (18 months) and lower (15 months) jaw.

Conclusion

The canine position and its significance from the functional and aesthetic perspective, fully justify orthodontic-surgical treatment in case of canines impaction. The potential aesthetic disadvantages of surgical treatment are related to gingival scarring and increase in the clinical crown length, which may require additional periodontal treatment. Orthodontic-surgical traction of impacted canines and their exposure and alignment into the dental arch exclude the need for prosthetic therapy and provide a nice smile to patients and proper occlusion.

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CASE REPORT

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Bridging psychological barriers between the child and the father after his returning from the war – Could group art therapy help?

Premošćavanje psiholoških barijera između deteta i oca posle očevog povratka iz rata – pomoć grupne *art* terapije

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Abstract

Introduction. War veterans with chronic post-traumatic stress disorder (PTSD) have poorer family and parenting functioning, but little research has focused on these impairments. **Case report.** This paper presented how the series of drawings and the group art therapy process enhanced bridging the psychological barriers of a 33-year-old male PTSD war veteran to engagement with the child. After two years of deployment he returned home and suffered mostly from PTSD numbness and avoidance symptoms. The veteran had the family readjustment difficulties and felt guilty for being detached from his 3-year-old son. He underwent integrative treatment in the Day Unit Program. The drawings series were made by free associations. Clinical observations and group discussions were recorded in the group art therapy protocols. The presented patient got gratifications and support from the group members for his illustration of popular cartoon heroes, and decided to draw Mickey Mouse at home. On the next session he shared his satisfaction for bridging the gap between him and his son, having done the same drawings with his son at home. Beck's depression inventory (BDI) was used for self-rating of depression and a reduction of BDI score from 18 to 6 during the treatment course was recorded. **Conclusions.** Series of drawings illustrated shift from war related past toward current family life of the war veteran. Group art therapy gave him gratification and support with hope and a sense of belonging, thus facilitated his parenting readjustment.

Key words:
stress disorders, post-traumatic; war; family; parent-child relations; art therapy.

Apstrakt

Uvod. Ratni veterani sa hroničnim posttraumatskim stresnim poremećajem (PTSP) slabije funkcionišu u porodici i kao roditelji, ali mali broj istraživanja se fokusira na ove disfunkcije. **Prikaz bolesnika.** Prikazano je kako su serija crteža i proces grupne *art* terapije pomogli u smanjivanju psiholoških barijera između ratnog veterana sa PTSP, starog 33 godine, i njegovog deteta. Nakon dve godine učešća u ratu vratio se kući sa PTSP simptomima, uglavnom emocionalnom otupelosti i izbegavanja drugih. Veteran je imao problema u prilagođavanju porodičnom životu i osećao je krivicu što nije blizak sa svojim trogodišnjim sinom. Uključen je u integrativni program lečenja u dnevnoj bolnici. Crteži su nastali prema slobodnim asocijacijama i izvršena je klinička opservacija serije crteža i protokola grupne *art* terapije. Dobio je pohvale i podršku zbog ilustrovanja popularnih crtanih likova od članova grupe i odlučio je da kod kuće crta Mikija Mausa. Na sledećoj sesiji nacrtao je Damba i pričao o zadovoljstvu zbog smanjenja distance između sebe i sina nakon što su zajedno crtali kod kuće. Na skali BDI (*Back Depression Inventory*) procenjena je blaga depresija na prijemu sa remisijom pri otpustu (skor 18 *vs* 6). **Zaključak.** Serija crteža prikazanog bolesnika ukazuje na promenu od ratne prošlosti ka trenutnom porodičnom životu. Grupna *art* terapija obezbedila je podršku i pohvale članova grupe ratnom veteranu i pružila mu nadu i osećaj pripadnosti, i olakšala ponovno prilagođavanje na roditeljsku ulogu.

Ključne reči:
stresni poremećaji, posttraumatski; rat; porodica; roditelj-dete odnosi; lečenje umetnošću.

Introduction

There are evidences that post-traumatic stress disorder (PTSD) can impair psychosocial and occupational functioning and overall well-being of veterans [1]. After war deployment PTSD is more prevalent in military veterans and higher rates of family reintegration problems were reported [2, 3]. In the frame of affected family cohesion and communications, military veterans often have reduction of attachment and parental satisfaction [4]. PTSD symptoms of emotional numbing

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and anger are particularly related to family relationships among military veterans and to providing the best treatment approaches interpersonal skills and family support are recommended [5]. Among the symptoms of combat-related PTSD emotional numbing, which consists of lack of experience feelings, detachment from others and lowering previously activities, together with avoidance present the hard-to-treat symptoms [6].

The findings suggest that family and social support play important role in the development, maintenance and recovery of PTSD [7]. Some researches support an integrative approach to intervention in which dealing with daily stressors and family problems give chance for treatment improvement [8]. Art therapy studies reported improvement with the various patients groups [9]. In war veterans, art therapy has been recognized as beneficial, effective and promising care, besides trauma-focused and other psychotherapy approaches [10].

The art group therapy in the frame of intergrative program in the Day Hospital of the Clinic for Psychiatry, Military Medical Academy, Belgrade, was described in this paper. This integrative treatment consisted of dynamically oriented open, heterogeneous group psychotherapy for neurotic- and stress-related disorders three times a week, recreational and occupational activities and individual counseling. The art group therapy had been applied once a week. The group was open, heterogenous with both geneder, civilian and military patients who were currently treated in the Day Hospital due to various mental disorders. This activity consisted of drawing by free association and free choice of coulored pencils and crayons on the same size of paper. In the same day the group session with exhibition, voting for the most interesting drawing and discussion of all drawings was performed for 90 minutes. After completion of the sessions the therapist conducted qualitative analysis of the drawings content, the form and the content of the group protocols.

This paper illustrated how the therapeutic effects of the group art therapy process can bridge the psychological barriers of veteran to engagement with his child and to gain perspective on their past and present life situations after war returning. Qualitative analysis of drawings and the issues from the group protocols that arise from group dynamics also performed through using art-based activities and discussion.

Case report

A 33-year-old married male military war veteran had returned from war in former Yugoslavia three months before his admission to non-specialize day unit treatment. He was referred after out-patient treatment of PTSD diagnosed according the DSM-IV criteria with dominant symptoms of re-experience and avoidance [11]. The history of other DSM-IV Axis I mental disorders was excluded. He was treated by counseling and pharmacotherapy and after four weeks of out-patient treatment the symptoms were decreased. He continued to take medications and returned to his job. However, next month his mental condition worsened and he was referred to day unit integrative treatment. On admission the patient gave written inform consent for participation in treatment, and ethic approval for drawing use was also obtained. The latest release of the Helsinki Declaration and principles of good clinical practice were applied.

The main complaints of the patient were decreased mood and guilt feelings due to avoidance his family members and the experienced detachment especially from his little son. During the two years of his participation in the war, he was separated from his family all the time, and only few times visited them shortly. But, now, after the war, at home, he yearned for rebuilding the lost connections with his family. Instead of that, in the afternoons he was sitting alone in the silence, staring only at TV, without saying anything to his wife and little son. He could not find the way to become closer to his 3-year-old son who was a 6-month-old baby when he was deployed. He was overwhelmed with grief because his son could not recognized him when he returned from the war.

On his first drawing the patient drew his flashback related to battlefield trauma (Figure 1). He named it as “Fear and silence before battle” and described his terrified experience with anticipation of death, horror, helplessness and grief for his killed friends. Next week he drew his military uni-

Fig. 1 – „Fear and silence before battle“.

form which he took off after returning home (Figure 2). He felt emptiness after war and ambivalence with a question what to do and how to continue and named the drawing "How to go ahead?" For these two drawings he got a few votes from other members. The next session brought him most voices for his drawing and was voted as most interesting (Figure 3).

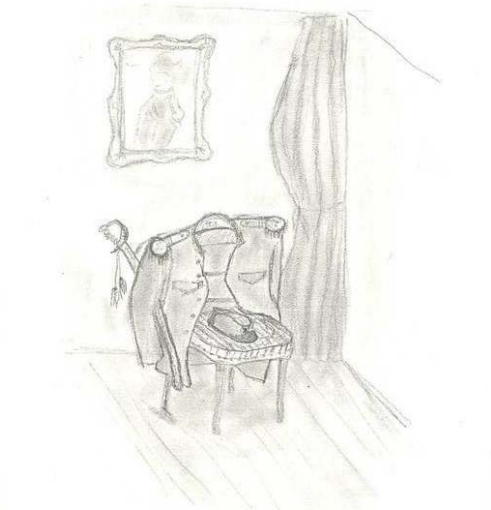

Fig. 2 – „How to go ahead?"

Fig. 3 – The childhood memories of Mickey Mouse.

He was surprised because he thought his drawing childless and felt ashamed. He had no idea what to draw at the beginning of the session, than he recalled that he liked to draw popular heroes of cartoons, and spontaneously, according his childhood memories drew Mickey Mouse. The members who voted for his drawing told that he made them very chilly and turned them to their happy childhood. They stressed that his drawings were made by great skills. He was very glad and next sessions he continued to draw other cartoon hero (Figure 4) and got many voices from the others and reported that he had already gotten most gratification from his son. He started drawing Mickey Mouse and other cartoon heroes at home. His son liked to watch him during drawing and tried to make drawing by himself. The father helped him and they drew together cartoon heroes on the walls of his bedroom. The patient became satisfied as parent and felt attached to his child and family. These feelings he shared with the group members on the verbal group sessions too.

The global functional assessment (GAF) scale was applied to measures psychological, social and occupational functioning [12]. Clinical assessment was performed and GAF score showed moderate symptoms on admission and mild on discharge (56 *vs* 68, respectively). The Beck's Depression Inventory (BDI) was used for self-rating depression and the reduction of the BDI score from 18 to 6 during the treatment course was recorded [13].

Fig. 4 – The elephant from a cartoom (free association).

Discussion

This case report presented with a series of drawings illustrated a course of positive change in the war veteran readjustment to family, especially to his little child. Qualitative analysis of the drawing content showed a shift from orientation from the war-related past toward the current family life. War veterans readjustment family problems such are feeling like a guest (40.7%), ambivalent about their family role (37.2%) and experience that their children were afraid of and detached from them (25.0%) were explored [6]. The series of these presented drawings illustrated crossover from war themes in the drawings to "here and now" problems of paternal impairments after a long war combat deployment. Military deployment is stressful not only for parents but also for their children [7]. A long deployment is related to challenges the parent and child distress [14]. There are a few clinical studies of impact of military deployment on young children [15]. The interrelation of family cohesion and PTSD symptoms was reported, however emotional sharing may moderate the PTSD effects on parental functioning [16–18].

Chronic post-deployment PTSD is often comorbid with depression and other mental disorders linked with prominent family readjustment problems [2]. In this case, the veteran had moderate impaired global functioning and mild depression on admission. On discharge his functioning was at the mild level impairment, and depression remitted. Other authors reported on the assessment war veterans by the GAF as a simple and useful tool [19]. There was reporting that PTSD veterans could overcome their treatment resistance and improve self-esteem and communication skills through group interaction in the creative atmosphere of art therapy [20].

Through artwork by free associations and regression the presented patient spontaneously recalled his favorite cartoon heroes from his happy childhood. A personal feeling of emotional numbing and social isolation which are commonly seen in patients with PTSD, were also reported in the presented case. The parenting role and communication with his only child, a little, 3-year-old son, became the focus of his day living. The group protocols from sessions contented the veteran's statement of feelings of emotional numbing and failure in parenting role. However, after the group session when members openly gave gratifications and supported him to continue drawing of chilly and favorite cartoon heroes, which he drawn by free associations, he got idea to do it at home. Very soon the gap between the father and his son was bridged. The veteran has been deployed for more than two years. His son was a baby when he went, and a 3-year-old boy when returned, home. In this age verbal communication has physiological and psychological limitations and drawing may enhance their self-expression.

The presented patient received a combined pharmacotherapy and integrative psychotherapy during participation to day treatment, so we cannot separately consider art therapy efficacy in PTSD treatment. However, this case was an illustrative example for a war veteran who suffered of chronic PTSD and improved his skills to cope with readjustment problems and to replace them from therapeutic setting to his family milieu.

Conclusion

The art group therapy could help war veterans to identify problematic interpersonal functioning and illustrate treatment response for each person through series of drawings. This treatment for war veterans PTSD may help to overcome avoidance and emotional numbing, especially related to parenting and reintegration into family.

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Redefinition of gender roles and fertility problems

Novo definisanje rodnih uloga i problemi plodnosti

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Ključne reči:
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Introduction

Modern day life along with the boom of cyber space communications has redefined a lot of fundamental concepts directly concerning character and partner relations. The quickness of expansion in information technology is constantly changing people's everyday lives. The unimaginable rate at which "time acceleration" [1] is going and the spread of communication space have started to threaten the classical parameters that connect people. Nowadays, almost all aspects of identity, being, closeness, family, intimacy and society are perceived and looked at from a new angle. The "fragmented" self in modern day living conditions does not necessarily indicate a psychiatric diagnosis, like personality disorder, but rather reflects a functional adaptation to altered living conditions brought about by the technological revolution. Amid the variety of choices and alternatives which are constantly offered to people in all spheres of life, ambivalence, confusion and indecisiveness have become "normal" not "pathological" categories. The new adaptive aim of a postmodern individual is "what to choose" among the vast number of equally acceptable alternatives, which are exponentially multiplying. Multitasking is taking its toll in the sense of frenetic communication as well as the lack thereof, a constant feeling of incompleteness and the chronic lack of "inner" space which could be used for integration of the experienced.

We could say that society is more and more fragmented in reality and organised in virtual space. There is the impression that people expect more from technology than from each other [2]. People are "alone together", often alienated from their environment, although they have a false sense of connection in virtual space, e.g. Facebook, Twitter. In the last 30 years, the average number of close people we can confide in has decreased from more than two to less than one person. In the digital era, personal integrity no longer has to be the ideal of a healthy personality and "multiphrenia", and the fragmented self [3] no longer have to be pathological phenomena, but rather functional adaptations of the fragmented world [4]. In these circumstances, many people often report feeling as if their time is being torn, frustrated and needing harmony. In addition, the liberalisation of life styles which have become accepted and are connected to politics of human rights, despite its advantages, create problems of multiple choices which in turn enable identity issues. In fact, aside from all the advances humankind is making with new technologies, at the same time there are problems emerging with the willingness of people to keep up with rapid changes and to adapt to them.

In this sense, the redefined social reality has particularly influenced partner relations, which have gone through some fundamental and "tectonic" changes only in the last few decades. Apart from the changing existential situation of each individual, a great shift in the definition of expected gender roles is also a factor of change in partner relations. According to definition "gender is social shaping of biological sex, defined by the accepted and acting roles assigned to men and women in society, in public and private life" (The European Council). Gender is a socially constructed definition, not only of women and men but also of the relations between

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women and men [5]. Unlike before, sex refers to biological characteristics and is defined primarily by the visible genitals and reproductive functions of a person, is established at birth and is socially assigned according to biological characters mentioned. Gender roles refer to "the characteristics of masculinity and femininity which enlighten the process of socialisation and psychological development" [6]. So, the term "gender" denotes the social construction of manhood and womanhood "of a specific society, in a specific time period, so assigned and therefore subjected to change" [7]. Gender roles are a multidimensional concept and refer to a wide spectre of features from attitudes and behaviours to personality traits. They are concerned with rules adopted through family upbringing, education, *via* media and general cultural content of a society. Gender identities arise through internalisation of social norms and are often closely connected with gender stereotypes, which contain specific principles of typical female and male features. Gender roles are therefore in relation to "traits that are assigned to men and women on the basis of presumed differences − characteristics and attributes, abilities and skills, respectively" [8].

In the last decades, however, prescriptive stereotypes that determine desired and appropriate behaviour for each gender, have suffered great changes as to which roles and responsibilities men and women should take. At the beginning of the 1970s, in correlation with the feminist social criticism, which was radical compared to the understanding of gender and sex of that time, a concept of androgyny emerges. Bem [9] introduces androgyny and defines it as a combination of masculinity and femininity. She considers these people to be the healthiest since none of the sets of traits dominate and so the person is easily adaptable to all situations. Since the position of women in social structures has started to rapidly change, so have the gender roles in the process of transition. Men and women start to take on similar roles in society; women postpone marriage and parenthood and spend more time on education and professional roles. Andocentrism ceases to dominate and so it is no longer believed that masculinity and acts by men are superior to femininity and acts by women. The impression that masculinity in women is more acceptable than femininity in men continues to diminish. The sexual revolution of the 1960s as well as the removal of homosexuality from the register of psychiatric disorders, represent signs of growing consciousness of the inevitability to tolerate individual differences in modern democratic societies, which has been an absolute imperative in the up-to-date times.

The redefinition of gender roles in Serbia

Serbian society could be perceived as a society between traditional and modern. Social, political and economic analyses indicate that in the last two decades, the modernisation of families has been put on hold to give way to "struggle for survival". As a result, social crisis has led to repatriarchalisation and retraditionalisation of gender roles [10] while the high migration rate from country to city areas has enabled the typically rural models to continue to exist in cities, thus changing the previous urban culture. Since Serbia is "a society in transition", the young generations are faced with the possibility of creating new gender identities which are, on one hand, heading towards reducing gender differences some of which have to do with attitudes, education and professional possibilities but on the other hand, increasing gender differences and the appearance of antagonism. The people interviewed perceive gender roles differently depending on whether they come from rural or urban backgrounds. The impression is that women who behave in a traditional manner are actually submissive, which leads to a lower level of well-being in a subjective sense. This means that masculinity dominates and is more desired which then stresses that masculine features such as activism, tendency towards leadership, are appreciated more in society, gain more social acknowledgement and contribute to higher self-esteem and better mental health).

As far as Serbian families are concerned, the most recent researches have shown that in most cases the traditional norm still dominates in which most housework is done by women, regardless of their professional engagements [11, 12]. Parent roles are also traditional and so the mothers are expected to be committed, permissive in upbringing, have a protective attitude towards children, while the primary role of the fathers is to be the financers and the breadwinners. It is interesting that both men and women perceive themselves as greater victims, both sexes consider that "they are worse off". It is considered that women with higher education are more satisfied with their life and there is a realistic increase in the position of women [13]. Family solidarity has been further strengthened by the transition period, despite gender imbalance in role perception. Also, there is an evident persistence in the traditional emotional attitude towards offspring in our society as well as in other Christian Orthodox societies. Characteristic of this attitude is the idealisation and the status of the parent ("mother" as a status symbol) on one hand and the status of the child on the other (the feeling of being "precious", putting the child on a "pedestal"), which is unlike the more pragmatic and rational approach that is characteristic of some other "Western" cultures. This in turn reflects on the parenting method and the preferred attachment rate which favour dependability and the presence of withness that often has the characteristics of a symbiotic relationship as well as significant ambivalence towards the possibility of separation. The difficult financial situation often serves as a good excuse and rationalises both sides' unwillingness to separate.

Considering that Serbian society is not only a transition society, but also a post-war society, along with all the negative effects following the war in the region. Examples of this are the "turbo folk" culture, growing of the incidence violence rate of all kinds and especially of domestic violence, the downfall of moral values and ethic principles etc. The young generations that were born or grew up in the war or post-war period, aside from transitional pressure, went through or are still going through the changed social expectations of what it means to be a man or a woman. The social models that were in effect only two decades ago can no longer be applied. The

new models are a combination of contemptuous rural-war identities mixed with global ideas about the identities of "some" Europeans which are considered "lucky" to be living in more prosperous surroundings and circumstances. Due to the inability to reach ideals of a comfortable or rich lifestyle young people fantasize about and which the media constantly describe as a life belonging to someone else, they start feeling emotional lethargy where it is "all the same" to them or they "let life take its course". In situations which they see as hopeless and pointless, they resort to temporary and easy "solutions".

What kind of effect does this have on partner relations? Fear of taking responsibility of any kind or degree was observed in partner relations. This can be referred to both sexes (same sex partner relations were not observed in this study) but is manifested differently in men and women. In men, there was a noticeable tendency not to accept the role of an adult especially when it came to starting a family while professionally, there was a noticeably tendency for immediate success and promotion while at the same time maintaining the status of someone who the primary family should continue to support in both a financial and organisational way. For example, even though they decide to start a family and have offsprings, they expect their parents to look after the children. The attention these men dedicate to their children depends on whether it will serve their narcissistic need for affirmation in their referential group with a typical example being that of a young father that spends time with his son or daughter by taking them to cafes where he can show them off to his acquaintances. This phenomenon can be termed as pseudo-independence, meaning to invest in a lifestyle which is only an imitation of adult life, with no actual responsibility taking. This style is nurtured by immaturity of character and so we have a generation of "late bloomers", individuals who do not emotionally "mature" before their 40, which is the time that they feel the need to start a family. Their motives are egocentric and more due to the fact that they want to make up for the lack of support, or symbiotic relationship, that they are getting from their primary family due to natural processes due to death, illness and such. These "forever young" men can be seen in groups similar to themselves as they spend their hours and days in cafes boasting about, if not their cars or scooters then gadgets, or their opinions on current affairs. They can take on different roles, whether that of political or sports experts, or of successful businessmen. Meanwhile, their girlfriends or wives, that is if they are in a committed relationship which a lot of them are not, and they do not see this as a problem, spend time in their subgroups, planning a shopping spree, a trip or going out to places where they will be seen and trying to define the type of woman they themselves would like to be among the various, mostly tabloid, types on offer. On the other hand, there are young people who lack positive role models in their surroundings and they move to other environments where they hope to achieve (and often do) high, strictly professional goals they set for themselves, at the cost of losing contact with their primary social environment from which they came from. In those new environments, they adapt to completely different gender roles and can never fit back in with the previous. There are of course, many more models of behaviour and experiences when it comes to young generations and here we have focused only on the ones which have a certain influence on redefining partner relations and relationships in our modern society and which have effect on family fertility.

The question is: How do young people create their intimate relations based on these models? What was observed in direct regards to our topic, was that the period in which both sexes start to show interest in starting a family and having offsprings is prolonged and often there is imbalance in this interest (only one side is interested while the other is passive). Also, it is difficult to perceive one's own incompetence for the "desired" role. All of this is evident in the way couples approach potential sterility treatment, which is not rare.

In the stated analysis *pro practis*, we see that in rural environments and with people of lower socioeconomic statuses or of lower education, conservative gender roles are present along with typical characteristics of traditional expectations. That is, takes place in the early reproductive period of life and family expansion is expected right after marriage has taken place, thus defining the primary role of a woman. This finding can be expected. In our analysis of the changing gender roles, of special interest was the second group of urban, educated couples, because that is where the change in traditional gender roles took place. As we can observe from the stated examples, the period of interest in family fertilisation is applied [14].

Expansion has been significantly moved back with the difference being over 10 years which constantly being pushed back even more. Aside from medical and psychological reasons for this shift, there are numerous socioeconomic and political reasons we will not go into, as they exceed the theme of our analysis. As far as medical reasons are concerned, what cannot be overlooked is the fact that a fascinating advance in medical interventions has led to "all being possible", which means that the boundary of realistic reproductive potential has shifted to a significantly later life age. Nikitović [15, 16] and Nikitović and Lukić [17] observed development tendency in Serbia as well as in other European countries. In the group of 27 countries of the European Union (EU-27), the average age of mothers at the birth of a child increased from 29.25 to 29.83 years in the period between 2003−2010. In Serbia, the increase in that same period was somewhat quicker, from 27.12 to 28.4 years but the average age of the mother at the birth of a child was still under the European Union average. The result of postponing giving birth, in regards to specific fertility age rates, presents a decrease in fertility rates in younger age groups, notably between 20 to 24 year olds, as well as an increase in the middle age groups, 30 to 39 year olds.

What we define as being psychological reasons, in regards to the couples' motivation for reproduction, is connected to their changed perception of themselves, partner relations, goals in life and towards global social issues. Furthermore, there is a strong impression that even in "later" years for reproduction, lots of women enrol in the programme without sincere motivation for offspring ("I wish to have a

child"), but because they are adapting to the social expectations or how they perceive these social expectations – typical sentences one might hear would be: "It's on the agenda now", "I'm in my late years", "It's time...". When one scratches under the surface of this statement, there is often evidence of great anxiety about becoming parents. It is often the women who are "motivated but frightened", while their partners who are often a few years older at this stage display less fear and greater, sincere desire. This is due to the lack of awareness that their life will change and also to traditional expectations in which the "mother is the most important" in the first few years of baby's life, and so they do not expect any great changes in their life after the arrival of the baby, which was proven to be true in reality. The only aspect men may have their doubts about is financial, although they are rare to admit it.

In more detailed individual interviews with women, the women were quick to give numerous honest answers in regards to the observed fear of pregnancy, which was in co-occurrence with great desire for pregnancy. In a few cases, this desire was "desire for pregnancy" that did not extend to "desire for a child" and which the interviewers were not aware of which is consistant with "narcissistic" culture. The fear referred to many spheres of being, depending on the structure of character, starting from body deformation and physiological changes which "cannot be controlled" to the loss of previous habits like going out, consumption of alcohol, travelling, exercising etc.; fear connected to raising the baby and the anticipating lack of partner's help; partner's fixed pattern of behaviour is often reported which includes an adolescent lifestyle – exercising, playing video games, drinking at night with friends, hanging out at cafes − "the immature" type, or partners which are constantly working and are hardly ever present − "the businessman" type; fears connected to the loss or maintenance of their position at work compared to new employees, should they take time off in their career and cannot keep up with professional challenges; fears connected to the family's existential future in light of the general economic crisis and perceiving their partner as incompetent, "immature", unmotivated, dependent; ambivalence and fears connected to primary families', both their own and partner's, involvement in their life, connected to assistance over the baby's upbringing; fears connected to the "definite" confirmation of their relationship with their partner with often couples being neither registered, nor married and making the "final" decision to commit to the partner they feel ambivalence towards, etc. There were a vast number of cases where the relationship was not primarily based on emotions but instead on rational decisions as to who might be the "adequate partner" for fulfilling the needs the person, usually the woman, has set. It is interesting that when a man is given such choices, he usually "picks" between acquaintances and former or current sexual-emotional partners, which woman would be the adequate choice "to be the mother of his children" and which would interfere the least with his up to then lifestyle, the so-called "narcissistic" choice and he is aware of this choice. In contrast, women in these years pick subconsciously, if they do not already have a partner and they often do not but choose a partner when they want to become mothers, partner which they can easily control, "the immature", dependent types, with a suggested reasoning being so that they could later complain about these traits.

Conclusion

It is characteristic for women to reject their traditional moulds and experiment with different models during their first stage of "acting out" their gender roles, i.e. during their 20s. On the other hand, in the second stage, i.e. 30s they "regress" into the traditional moulds and this occurs mainly with the reproductive function. Despite many changes and redefinitions of gender roles in the contemporary society, when faced with the proverbial biological clock, many women quickly regress into their traditional roles despite rejecting them beforehand. Doing so, they often forsake an authentic choice of partners in favor of birth being a guarantee. Along with this, the attitudes of gynaecologists have changed, as well from an earlier definition that a marriage is sterile after two years of the couple living together with regular intercourses to today's attitude that it is after only one year of the couple living together with regular intercourses before proclaiming the marriage to be sterile. Earlier testings would last for up to a year or two, with all the testings being completed with three to four months today. Thus, the decision for active therapy and aided fertilisation comes earlier.

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I N M E M O R I A M

prof. dr
ZORAN ROGANOVIĆ
pukovnik u penziji
(1958–2016)

Posle kratke i teške bolesti, dana 9. 6. 2016. godine, u Beogradu je preminuo pukovnik u penziji, prof. dr Zoran Roganović, doskorašnji načelnik Klinike za neurohirurgiju Vojnomedicinske akademije (VMA), u Beogradu, redovni profesor hirugije (podoblast neurohirurgija) Medicinskog fakulteta VMA Univerziteta odbrane u Beogradu, izuzetan čovek, hirurg i pedagog, poznat i van granica naše zemlje.

Profesor Roganović rođen je 27.9.1958. godine u Prilužju, a osnovnu školu i gimnaziju završio je u Kraljevu. Medicinski fakultet Univerziteta u Beogradu završio je 1982. godine sa prosečnom ocenom 9,75. Još za vreme studija pokazao je veliko interesovanje za neurohirurgiju, a za radove izlagane na studentskim kongresima i pokazane rezultate u savladavanju redovnog programa na fakultetu više puta je nagrađivan od strane matičnog fakulteta i univerziteta.

Posle završetka obaveznog lekarskog staža i dve godine rada u hitnoj službi Medicinskog centra Kraljevo započinje specijalizaciju iz neurohirurgije na VMA i završava je juna 1991. sa odličnom ocenom. Nakon završetka specijalizacije raspoređen je na mesto lekara specijaliste u Klinici za neurohirurgiju, gde 2000. godine postaje načelnik 1. odeljenja. Za načelnika Klinike za neurohirurgiju VMA postavljen je 2006. godinuei tu funkciju obavljao je sve do juna 2016. godina, kada zbog naglog pogoršanja zdravstvenog stanja, odlazi u prevremenu penziju.

Magistarski rad „Kongenitalna anomalija krvnih sudova mozga (osim aneurizme)“ odbranio je 1993. na VMA. Tokom rata na području prethodne Jugoslavije znatno je doprineo zbrinjavanju ranjenika kako na VMA, tako i na ratištu, gde je boravio aprila i maja 1992. Posebno se interesovao za povrede perifernih nerava o kojima su podaci u kliničkoj literaturi bili veoma oskudni. Zajedno sa kolegama iz Klinike operisao je u trogodišnjem periodu oko 1 500 ranjenika sa povredama perifernih nerava, pri čemu je u Klinici i na ovim prostorima inaugurisao novu, a u svetu veoma aktuelnu tehniku premoštavanja perifernih nerava denaturisanim mišićnim graftom. Ovakav angažman rezultirao je izradom doktorske disertacije sa temom „Faktori koji utiču na ishod operativnog lečenja ratnih neurotmeza“, koju je odbranio na VMA februara 1995. godine. Pored zbrinjavanja ranjenika, učestvovao je sve vreme u izvođenju elektivnih operacija na mozgu, pre svega cerebrovaskularne patologije i patologije pontocerebelarnog ugla.

Profesor Roganović usavršavao se u najpoznatijim evropskim neurohirurškim centrima. Tokom 1991. godine bio je na stručnom usavršavanju u Ljubljani kod poznatog neurohirurga Vinka Dolenca, a u periodu 1994–2005. u više navrata boravio je u čuvenim kliničkim centrima u Velikoj Britaniji, Nemačkoj i SAD.

Rezultate svog profesionalnog rada predstavljao je na brojnim kongresima i simpozijumima širom sveta, često kao predavač po pozivu. Objavio je više od 150 stručnih i naučnih radova, od kojih veći broj u vodećim svetskim neurohirurškim časopisima, 3 knjige (jedna izdata u inostranstvu) i 13 poglavlja u domaćim knjigama. Bio je rukovodilac i tri

naučnoistraživačka projekta (o proceni aktivnosti ateroskleroze karotidnih arterija, o rekonstrukciji kranijalnih defekata i o neurohirurškom lečenju lumbalnih bolova) iz kojih su i proistekli mnogi od ovih saopštenja.

Uporedo sa izuzetno zahtevnim stručnim i naučnoistraživačkim angažmanom, profesor Roganović bio je veoma aktivan i na polju obrazovanja. Od 1996. godine, kada je izabran u prvo nastavno zvanje docent (2001. vanredni profesor, a 2006. redovni profesor), aktivno je učestvovao u izvođenju nastave u Školi rezervnih oficira sanitetske službe, na specijalističkim i poslediplomskim studijama na VMA, a od 2009. godine, kada je počela sa radom Visoka škola VMA, kasnije Medicinski fakultet VMA, i na integrisanim akademskim studijama medicine, kao i doktorskim studijama na tom fakultetu.

Pored nabrojanih funkcija i obaveza, treba pomenuti i učešće profesora Roganovića u brojnim stručnim telima VMA, Medicinskog fakulteta VMA i Univerziteta odbrane. Posebno treba istaći njegovu funkciju zamenika, kasnije i zastupnika načelnika Grupe hirurških klinika VMA (2012) i predsednika Saveta Medicinskog fakulteta VMA (2011).

Profesor Roganović je bio član brojnih stručnih neurohirurških udruženja u zemlji i inostranstvu (član Udruženja neurohirurga Srbije i Srpskog lekarskog društva, član Komiteta za periferne nerve Svetske neurohirurške asocijacije, rukovodilac naučnog odbora AO *Spine* udruženja, član Američkog udruženja vojnih hirurga, član Udruženja neurohirurga Engleske, Svetske federacije neurohirurških društava i Evropske asocijacije neurohirurga). Kao ekspert u svojoj oblasti često je bio angažovan u svojstvu recenzenta od strane redakcija brojnih domaćih i međunarodnih medicinskih časopisa. S ponosom ističemo da je profesor Roganović bio član uredništva „Vojnosanitetskog pregleda“ (VSP) upravo u periodu kada je časopis ušao u sistem praćenja čuvene citatne baze *Science Citation Index Expanded* (SCIe) i dobio svoj prvi impakt faktor, i da je njegovo angažovanje kao autora, urednika i recenzenta VSP-a sigurno doprinelo tom uspehu. I ne samo to, profesor Roganović je bio dva puta dobitnik priznanja Autor godine VSP, 1995. i 1996, priznanja koje izdavač i uredništvo časopisa dodeljuju autoru koji u godini za koju se dodeljuje priznanje objavi najviše radova u VSP-u, pri čemu se prednost daje onome tko, kao jedini ili prvi autor, objavi najviše originalnih radova. Profesor Roganović ostaće zabeležen u istoriji našeg časopisa kao prvi dobitnik tog priznanja.

Kao lekara i humanistu krasile su ga najplemenitije osobine, zbog kojih će trajno ostati u sećanju svojih pacijenata, kolega, saradnika i studenata.

Njegovim preranim odlaskom izgubili su svi, u prvom redu njegova porodica, ali i svi mi koji smo ga poznavali. Mogao je još mnogo toga da pruži medicinskoj struci i nauci, iako je već ostavio dubok trag, ne samo u medicini Srbije, već, slobodno se može reći, i svetskoj medicini.

Zbog toga, neka mu je večna slava i hvala!

prof. dr Silva Dobrić
glavni i odgovorni urednik VSP

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Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

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