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ORIGINAL ARTICLES / ORIGINALNI RADOVI

Dr Roman Sondermajer (28. februar 1861 – 30. januar 1923) je bio hirurg, sanitetski pukovnik i načelnik saniteta Vrhovne komande Srbije 1916–1917. Smatra se rodonačelnikom srpske ratne hirurgije. Svojim radom i zalaganjem doprineo je unapređenju srpskog vojnog saniteta, preveo je i prilagodio našim prilikama Ratnu sanitetsku službu austrijske vojske, izvršio modernizaciju i popunu ratne sanitetske opreme. Reorganizovao je sanitet srpske vojske na Krfu i u Solunu tokom Prvog svetskog rata.
Ove godine, 28. februara, navršilo se 148 godina od njegovog rođenja.

Dr. Roman Sondermayer (February 28, 1861 – January 30, 1923) was a surgeon, medical colonel and a head of the Medical service of the Supreme Command of Serbia during 1916–1917. He is considered to be the mayor of Serbian war surgery. Through his work and dedication, he contributed to the improvement of the Serbian military medical care, translated and adapted the Military Medical Service of the Austrian Army to our circumstances, and modernized and completed the war medical equipment. He reorganized the medical service of the Serbian Army in Corfu and Thessaloniki during the First World War.
This year, on February 28th, 148 years passed since he was born.

ORIGINAL ARTICLES

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Prevalence of dental caries among the children from the SOS Children's Village in Croatia

Prevalenca karijesa kod dece SOS Dečjeg sela u Hrvatskoj

Zrinka Ivanišević*, Marko Matijević*, Zvonimir Užarević†, Djordje Petrović‡, Davor Jurlina§, Vjekoslav Jerolimov*

University of Osijek, *Faculty of Dental Medicine and Health, †Faculty of Education, §Faculty of Medicine, Osijek, Croatia; University of Novi Sad, ‡Faculty of Medicine, Novi Sad, Serbia

Abstract

Background/Aim. Oral health is an integral part of general health and an important factor in the overall quality of life. The purpose of this study was to investigate the prevalence of dental caries among the children from the SOS Children's Village in Croatia. **Methods.** The dental examinations based on the World Health Organization criteria were performed on 88 children from SOS Children's Village in Croatia. The teeth were clinically examined with standard dental instruments using the visual-tactile method under standard light. The clinical indexes of decayed, missed, and filled (dmft and DMFT, for primary and permantnt teeth, respectively) and decayed, missed, and filled surfaces (DMFS), as well as the significant caries index (SiC) were recorded. **Results.** Among the children from the SOS Children's Village caries incidence were 57.94%. The mean dmft, DMFT and DMFS of all children was 1.82, 1.90 and 2.82, respectively. The highest mean dmft and DMFT score of 4.24 and 2.56 was found among 7–10 and 11–14 years old children, respectively. The highest mean DMFS score of 3.85 and 3.90 was found among 11–14 years old children and among the children from the SOS Children's Village Lekenik, respectively. Among all children, the SiC index was 4.69. There was a significant difference between age groups and children's place of residence in DMFT, DMFS and SiC. **Conclusion.** Prevalence of dental caries is low among the children from the SOS Children's Village in Croatia compared to the children who lived with biological families.

Key words:
dental caries; child; child, preschool; child abandoned; dmf index; prevalence; croatia.

Apstrakt

Uvod/Cilj. Oralno zdravlje je integralni deo opšteg zdravlja i predstavlja važan faktor kvaliteta života. Cilj rada bio je da se istraži prevalenca zubnog karijesa kod dece iz SOS Dečjeg sela u Hrvatskoj. **Metode.** Zubni pregledi na osnovu kriterijuma Svetske zdravstvene organizacije su sprovedeni kod 88 dece iz SOS Dečjeg sela u Hrvatskoj. Zubi su klinički ispitani standardnim stomatološkim instrumentima pomoću vizuelno-taktilnog načina pod standardnim svetlom. Zabeleženi su klinički indeksi: indeks karioznih, ekstrahovanih i plombiranih zuba (KEP) (za mlečne zube i za stalne zube), indeks površina karioznih, ekstrahovanih i plombiranih (DMFS) zuba i značajni kariozni indeks (SiC). **Rezultati.** Kod dece iz SOS Dečjeg sela incidencija karijesa bila je 57,94%. Prosečna vrednost KEP indeksa za mlečne zube iznosila je 1,82, KEP indeksa za stalne zube 1,90 i vrednost DMFS indeksa 2,82. Najviše prosečna vrednosti KEP indeksa za mlečne zube od 4,25 registrovane su u grupi dece od 7–10 godina starosti a KEP indeksa za stalne zube od 2,56 u grupi dece od 11–14 godina starosti. Najviše prosečne vrednost DMFS indeksa registrovane su u grupi dece od 11–14 godina starosti (3,85) i kod dece iz SOS Dečjem selu Lekenik (3,90). Prosečna vrednost SiC indeksa za svu pregledanu decu iznosila je 4,69. Postoji značajna razlika između starosnih grupa dece i dece s obzirom na mesto prebivališta u vrednostima KEP, DMFS i SiC indeksa. **Zaključak.** Prevalencija zubnog karijesa je niža kod dece iz SOS Dečjeg sela u Hrvatskoj u poređenju sa decom koja žive u biološkim porodicama.

Ključne reči:
zub, karijes; deca; deca, predškolska; deca, napuštena; dmf indeks; prevalenca; hrvatska

Introduction

Oral health is an integral part of general health and an important factor in overall quality of life. Despite great efforts to preserve oral health, not only in Croatia but also all over the world, oral cavity diseases are on the rise [1, 2]. Dental caries is the most common infectious disease of the oral cavity. Numerous factors have an effect on the appearance of

Correspondence to: Zvonimir Užarević, University of Osijek, Faculty of Education, Cara Hadrijana 10, 31 000 Osijek, Croatia.
E-mail: zuzarevic@foozos.hr

caries: gender, age, socioeconomic status, cultural and religious factors, environmental factors as well as diet and oral hygiene habits [3–5]. Dental caries is the most common chronic disease in children - five times more common than asthma and seven times more common than seasonal allergies in children. In addition, dental caries is the fourth most expensive disease to treat in the third world countries [6, 7]. One of the most important tasks of the health profession is to prevent dental caries. Due to differences in enamel structure, inadequate oral hygiene or lack of preventive measures, caries of primary teeth is more common than in permanent teeth [8]. Measures of caries prevalence are indexes of decayed (D), missed (M), and filled (F) permanent teeth (T) or surfaces (S), i.e., DMFT and DMFS indexes, and decayed (d), missed (m), and filled (f) primary teeth (t) or surfaces (s), i.e., dmft and dmfs indexes [9].

Taking care of oral health is the task of society and the family children are raised in. Considering the 132 million children without parents around the world, there is a need for various forms of care, which along with the basic needs of children meet their economic, psychosocial and health needs [10]. They are especially vulnerable group that needs particular attention. The life of children growing up in institutional care is usually devoided of an enabling environment which often leads to a complex mixture of physical, perceptual, social, intellectual and emotional deficits and can result in a deficiency of cognitive, social and physical as well as medical well-being [11].

The SOS Children's Villages is the leading global organization in the field of alternative care for children. The first SOS Children's Village was founded in 1949 in Imst, Austria. Today, The SOS-Kinderdorf International is the leading organization for 133 countries, with 533 SOS Children's Villages. A typical SOS Children's Village has 15 houses, a kindergarten and a community center that is accessible to the local community. SOS Children's Villages operate under the United Nations Convention on the Rights of the Child, promoting this right around the world. Child development in a caring family environment is supported through realization of the following rights: welfare, education, health (preventive and active health care) and psychosocial support. The SOS Children's Villages in Croatia have been working for 20 years in two SOS Children's Villages, Ladimirevci and Lekenik. In SOS Children's Villages, the SOS mothers play a crucial role in the lives of children, providing them a home and stable family environment. The SOS mothers undergo a careful selection process and long-term training and meet all the physical and emotional needs of their children [12, 13]. One of the elements of a healthy psychological and physical development of children is care of oral health; nevertheless specific conditions of life in this environment (child – SOS mother) can, at an early age, affect not only the physical and mental growth and development but also the state of oral health. In published literature, there is very little data about oral health of children in this population and there is generally a lack of data on oral health of children in SOS Children's Villages in Croatia.

The aim of this study was to determine the incidence of dental caries among primary school children in the SOS Children's Villages in Croatia, Ladimirevci and Lekenik, using the indexes for decayed, missed, and filled teeth/surfaces for primary and permanent teeth (dmft, DMFT, and DMFS, respectively) and significant caries index (SiC). This research will contribute to new knowledge about oral health of this population of children who will eventually be used as a starting point in planning the necessary preventive and educational measures in the future.

Methods

Participants

The study was conducted during 2015 in the SOS Children's Villages in Croatia, Ladimirevci and Lekenik, where 30 SOS mothers cared for about 250 children of early, primary and secondary school age. According to the regulation of admission in the SOS Children's Village, the SOS families receive children without major developmental difficulties, up to 10 years of age, or older, if they are biological family with more brothers and sisters who come to the SOS Children's Village together. In this study, 88 primary school children were included (46 boys and 42 girls), from both SOS Children's Villages, at the age of 7 to 14 years (the average age was 11.45 ± 2.22 years) for whom a signed consent of their biological parents was obtained. To conduct this research, the license of the Ethical Committee of the Faculty of Medicine at University of Osijek was obtained (Class: 602-04/13-08/09; No. 2158-61-07-13-45; Date: 16 December 2013).

Procedure

The children were examined in dental practice under standardized conditions of the World Health Organization (WHO), in terms of controlled hygiene with appropriate lighting. The medical examinations were conducted by a single experienced examiner with the help of an assistant who recorded the data of the oral status in prepared forms made according to the WHO method from 1997 [14]. Calibration of the examiner was done in a way that she examined 30 children of different age two months before and immediately before the study in which the kappa value was 0.95. The clinical research approach was the same for all children and it included a visual and tactile inspection of oral cavity with a probe, mirrors and syringe [15, 16]. Prior to the clinical examination and evaluation of oral status, teeth were cleaned of soft and hard deposition and plaque using a rotating brush and prophylactic paste without fluoride. Then teeth were rinsed with water and air, dried, and a dry working field was secured using the saliva ejector and cotton rolls to isolate teeth from buccal/labial mucosa and tongue. Every tooth (or surface) which by probing and visual inspection showed signs of lesions in pits, fissures or walls (cavities, undermined enamel, finding of soft walls) was recorded as carious. The evaluation was determined according to the WHO criteria, and information about each tooth was recorded using the standard codes [14]. All examined teeth were included in the calculation of the final dmft (total number of decayed, missing or filled primary teeth), DMFT (total number of decayed, missing or filled permanent teeth), DMFS (total number of decayed (D), missing (M) or filled (F) permanent teeth surfa-

ces) and SiC (the mean DMFT for one third of the population with the highest caries scores) indexes [17]. The indexes were compared between the male and female participants, place of residence and age groups.

Data analysis

The Microsoft Office Excel 2007 for Windows (Microsoft Corporation, USA) was used for the entry of data on oral health status and for creating the tables. The data were statistically processed using the statistical package Statistica 12 (StatSoft, Inc., USA). The level of significance was set at 5%.

Results

The study included a total of 88 participants from two SOS Children's Villages in Croatia, Ladimirevci (53.41%) and Lekenik (46.59%); among them, there were 52.27% boys and 47.73% girls whose average age was 11.45 ± 2.22 years. The age group of 7–10 year olds accounted for 32.95%, and the age group of 11–14 year olds accounted for 67.05% of participants (Table 1). Mixed dentition was present in 52.27%, permanent in 47.73% of children whereas there were none with primary dentition.

From the total number of children included in the study, 57.94% of them had carious changes on examined teeth. The carious changes were more frequent in the children from the SOS Children's Village Lekenik (63.41%) compared to the children from the SOS Children's Village Ladimirevci (53.19%) and in the children aged from 11–14 years (74.58%) compared to children aged from 7–10 years (24.14%). The presence of caries lesions in relation to gender was equal in both groups (about 58%) (Table 1).

The mean values of the DMFT and DMFS indices with respect to the gender did not differ significantly and amounted to 1.72 ± 1.99 and 2.61 ± 3.14, respectively for boys and 2.10 ± 2.68 and 3.10 ± 4.23, respectively for girls. The significant differences in the mean values of the DMFT and DMFS indices were observed among the groups of children with regard to the place of residence and amounted to 1.49 ± 7.2 and 2.09 ± 2.98, respectively for the children from Ladimirevci and 2.37 ± 2.55 and 3.90 ± 7.5, respectively for the children from Lekenik. Also, a significant differences were revealed between the mean values of the DMFT and DMFS indices with respect to the age group to which children belong and it amounted to 0.55 ± 1.24 and 0.72 ± 1.71, respectively for children aged 7–10 years and 2.56 ± 2.47 and 3.85 ± 3.93, respectively for children aged 11–14 years. The mean values of the DMFT and DMFS indices for the total sample of participants amounted to 1.90 ± 2.33 and 2.82 ± 3.66, respectively. The largest share of the DMFT and DMFS indices made the D-component with 62.63% and 60.88%, respectively followed by teeth with fillings (33.69% and 36.53%, respectively) and extracted teeth (3.68% or 2.59%, respectively) (Tables 1 and 2).

The SiC index for the total sample of participants was 4.69 showing a significant statistical difference between the value of SiC index based on the place of residence (3.63 for Ladimirevci and 5.36 for Lekenik), as well as with regard to a certain age group (1.60 for the children aged 7–10 years and 5.45 for the children aged 11–14 years) (Table 1).

Table 1

Mean values and standard deviations (SD) of dmft, DMFT, DMFS and SiC index related to gender, place of residence and the age group of children from the SOS Children's Village in Croatia

Parameter	Number of participants n (%)	dmft (mean ± SD)	DMFT (mean ± SD)	DMFS (mean ± SD)	SiC (mean)	Without caries n (%)
Gender						
male	46 (52.27)	2.24 ± 3.33	1.72 ± 1.99	2.61 ± 3.14	–	20 (43.48)
female	42 (47.73)	1.29 ± 2.61	2.10 ± 2.68	3.10 ± 4.23	–	17 (40.48)
SOS village						
Ladimirevci	47 (53.41)	1.96 ± 3.04	1.49 ± 2.07	2.09 ± 2.98	3.63	22 (46.81)
Lekenik	41 (46.59)	1.66 ± 3.05	2.37 ± 2.55*	3.90 ± 5.07*	5.36*	15 (36.59)
Age group (years)						
7–10	29 (32.95)	4.24 ± 3.57	0.55 ± 1.24	0.72 ± 1.71	1.60	22 (75.86)
11–14	59 (67.05)	0.63 ± 1.79**	2.56 ± 2.47**	3.85 ± 3.93**	5.45**	15 (25.42)
Total	88 (100)	1.82 ± 3.03	1.90 ± 2.33	2.82 ± 3.66	4.69	37 (42.06)

dmft – decayed, missed and filled primary teeth; DMFT – decayed, missed and filled permanent teeth; dmfs – decayed, missed and filled surfaces of primary teeth; DMFS – decayed, missed and filled surfaces of permanent teeth; SiC – significant caries.

*** – significant differences related to the place of residence ($p < 0.05$); ** – significant difference related to the age group affiliation ($p < 0.05$).**

Table 2

Mean values of carious (dt/DT/DS), extracted (mt/MT/MS) teeth and teeth with fillings (ft/FT/FS) related to gender, place of residence and the age group of children from the SOS Children's Village in Croatia

Parameter	dt	mt	ft	DT	MT	FT	DS	MS	FS
Gender									
male	1.52	0.43	0.33	1.16	0	0.59	1.63	0	0.98
female	0.85	0.34	0.10	1.26	0.15	0.71	1.85	0.15	1.10
SOS village									
Ladimirevci	1.79	0.06	0.11	1.28	0	0.21	1.85	0	0.23
Lekenik	0.54	0.76	0.41	1.10	0.15	1.12	1.59	0.15	1.93
Age group									
7–10	2.69	0.90	0.66	0.38	0	0.17	0.45	0	0.28
11–14	0.47	0.14	0.05	1.59	0.10	0.86	2.36	0.10	1.39
Total	1.22	0.40	0.22	1.19	0.07	0.65	1.76	0.07	1.05

dt/DT/DS – decayed primary/decayed permanent/decayed surfaces of permanent; mt/MT/MS – missed primary/missed permanent/missed surface of permanent; ft/FT/FS – filled primary/filled permanent/filled surface of permanent.

Discussion

With this study we got an insight into the incidence of dental caries, values of dmft, the DMFT, DMFS and SiC indices among the population of primary school children (7–14 years old) in the SOS Children's Village in Croatia. As data on the above indices for other SOS Children's Villages in the world are missing, with the exception of the research conducted in the SOS Children's Village Bhopal in India, our results can be compared with the results of the monitored parameters only among the general population of children and children living with biological parents. The prevalence of caries in the survey conducted among the children of the SOS Children's Villages in Croatia amounted to 57.94%, which is the lower value compared to the value recorded in the study conducted among the twelve-year old children in Montenegro (88.35%) and in relation to the value recorded in Romania among children population aged 10–17 years (75%) [18] as well as in a group of children aged 11–13 years (83.1%) [19]. The higher values of the prevalence of dental caries were recorded among 12 year olds in Greece as well (63%) [20]. The lower values of the prevalence of dental caries in relation to our population were recorded among 12 years old children from Cyprus (32.6%) and Germany (31%) [21].

The mean values of the dmft, DMFT and SiC indices recorded in the conducted research was in the range of recommended values of the WHO regarding oral health of 12 years old children. In European countries, the average dmft index for children aged 5–7 years ranges from 0.9 to 8.5. In this study, which involved group of children aged 7–10 years, the dmft index was 4.24. The lowest values of the dmft index were recorded in Spain (1.0) and Denmark (1.3). Children in Finland, Netherland and Norway also had the mean values of dmft index below 2.0. The lowest value of the dmft index of 0.9 was recorded in Ireland [22].

The data of the incidence of dental caries in the children population of other SOS Children's Villages in the world are insufficient. The only known research results are related to the SOS Children's Village Bhopal in India, where children from infancy to the age of 20 years were included. The results of that study showed that the value of the DMFT index was observed within the age group of 11–15 year olds amounted to 2.9 [23], whereas in our study, among the same age group of children, the DMFT index was 2.56. Among the age group of children from 6–10 years from the SOS Children's Village Bhopal, the DMFT index was 0.17 as a lower value with respect to the registered DMFT index within the same age group of children in our study (0.55). The values of the dmft/DMFT index for the whole tested population of children in the SOS Children's Village Bhopal was 0.31/1.03 which is slightly lower value compared to the dmft/DMFT referring to the population of children in the SOS Children's Village in Croatia [23].

The results of the study conducted among the population of children aged 7–14 years who lived in families of urban centers (Zagreb, Croatia) show value of the DMFT index 4.1, which is a higher value in relation to the population of children of the same age group from the SOS Children's Village in Croatia. Furthermore, the value of DMFT index within children aged 11–14 years, who live in families of urban centers (Zagreb, Croatia), was 5.9, which is also a higher value in relation to the same age group of children from the SOS Children's Village in Croatia. The SiC value index for the population of children living in biological families of urban centers (Zagreb, Croatia) amounted to 7.4 [2], whereas it was lower among the population of children from the SOS Children's Village in Croatia (4.69). Results of a study conducted among population of children aged 3-14 years in rural and sub rural areas of central Croatia showed a higher value of dmft/DMFT (7.7/6.7) and the SiC index (10.89) when

compared to the children from the SOS Children's Village in Croatia [24].

Comparing the results of the dmft/DMFT index (4.24/2.56) from our study with the results of neighboring Bosnia and Herzegovina, it is visible that 6 years old children in Bosnia and Herzegovina had the higher values of the dmft index (6.71) as well as the values of DMFT index (4.16) for 12 years olds [25]. In contrast to the above studies, a survey conducted among primary school children aged 6–11.5 years in Slovenia showed a similar value of the DMFT index (0.66) to the children from the SOS Children's Village in Croatia (0.55). The value of the dmft index in the above-mentioned Slovenian research was 2.83 which is a smaller value compared to the value of the dmft index (4.24) recorded among the children from the SOS Children's Village in Croatia [26].

The mean values of DMFT index in most countries is below 3, and in the countries of north-western Europe and the United States is even below 2 [27]. However, other European population of children, especially those children who lived in the Mediterranean region showed different values of the DMFT index. Twelve-year old Sicilian children had a mean DMFT index of 2.88 and their peers in Sardinia 2.4 [28, 29]. Twelve-year old children in Greece had the DMFT index of 2.77 to 6.74 [30]. Among 12 years old children in Spain, the mean DMFT index was 1.33, with the tendency to fall below 1 by the end of 2015 [31, 32]. In their study, Almerich-Silla and Montiel-Company [33] recorded the mean DMFT index of 2.43 for 12 years old immigrant children and 0.99 in the domicile Spanish children. The mean value of the DMFT index among 8 and a 9 years olds in Germany was 0.7, and in Hungary 0.4 [34]. On the other hand, 12 years old children in many countries had a mean value of the DMFT index greater than 3, as was the case in Latvia (7.7), Poland (5.1), Ukraine (4.4), Hungary (4.3), Lithuania and Belarus (3.8), Russia (3.7) [22]. Also, the values of the DMFT index for children from Montenegro and Kosovo amounted to over 3 [35, 36]. The results of this research among children in the age group of 7–14 years showed lower values of the DMFT index (1.90) comparing to the previously mentioned studies.

Conclusion

The relatively low total value of the DMFT index in this study is likely to be a result of the care of the SOS mothers and caring family environment and continuous education about the importance of oral health. Continuing education about the importance of oral health and welfare institutions in which the children live, as well as the high awareness of the importance of oral health that these children have, might be a possible reason for relatively low values of the DMFT index. The specificity of life of the children in the absence of biological parents but in a caring family environment (child – SOS mother) results in better individual approach and commitment to oral health of each individual child. A high level of the awareness of oral health is present in the children and SOS mothers in the SOS Children's Villages in Croatia should be a model and a starting point in planning and implementation of further preventive and educational measures for the population of children who grow up with biological parents.

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Attachment and emotional regulation in adolescents with depression

Afektivno vezivanje i emocionalna regulacija kod adolescenata sa depresijom

Željka Košutić, Marija Mitković Vončina, Vesna Dukanac, Milica Lazarević, Ivana Raković Dobroslavić, Mina Šoljaga, Aleksandar Peulić, Mina Djurić, Danilo Pešić, Zagorka Bradić, Dušica Lečić Toševski

Institute of Mental Health, Belgrade, Serbia

Abstract

Background/Aim. Attachment and emotion regulation skills are recognized as important factors in the development of depression, but their specifics have rarely been discussed in clinical adolescent population. The aim of our study was to investigate attachment and emotion regulation strategies in adolescents with depression. **Methods.** The sample consisted of 101 adolescents, age 16 to 24, divided into three groups: 1) 41 adolescents with the diagnosis of depressive disorder; 2) 30 adolescents with the diagnosis of anxiety disorder; 3) 30 health adolescents (without psychiatric diagnosis). The assessment was done by the following instruments: the Socio-demographic questionnaire; the Semistructured clinical interview (SCID-I) for the Diagnostic and Stratistical Manual of Mental Disorders, Fourth Edition (DSM-IV); the Beck Depression Inventory (BDI); the Inventory of Parent and Peer Attachment (IPPA) and Emotional Regulation Questionnaire (ERQ). Data were analyzed using MANCOVA and partial correlation, with gender, age and birth order as covariates. **Results.** The adolescents with depressive disorders had less secure attachment to mother and peers than the health adolescents and less secure attachment to father comparing to other two groups (MANCOVA F = 4.571; $p = 0.000$). The adolescents with anxiety disorder had less secure attachment to father and peers compared to the healthy adolescents group ($p < 0.05$). The depressed adolescents used the strategy of cognitive reappraisal less often than both control groups (MANCOVA F = 5.200; $p = 0.001$). Subjective experience of depressive symptoms was related to insecure attachments to both parents and peers (r = -0.457; -0.436; -0.349; $p = 0.000$), as well as to lower use of cognitive reappraisal (r = -0.446; $p = 0.000$). **Conclusion.** Our findings related the adolescent depression to insecure attachment in all domains, with the specific weakness in emotion regulation (weak cognitive reappraisal). The findings could have practical implications for preventive and therapeutic interventions.

Key words:
adolescent; depression; emotions; object attachment.

Apstrakt

Uvod/Cilj. Afektivno vezivanje i veštine emocionalne regulacije prepoznati su kao značajni faktori u nastanku depresije, ali su retko razmatrane njihove specifičnosti u adolescentnoj kliničkoj populaciji. Naše istraživanje imalo je za cilj da ispita razlike u afektivnom vezivanju i strategijama emocionalne regulacije kod adolescenata sa depresijom. **Metode.** Uzorkom je bio obuhvaćen 101 adolescent, starosti 16 do 24 godine, od toga 41 adolescent sa dijagnozom depresivnog poremećaja, 30 adolescenata sa dijagnozom anksioznog poremećaja i 30 zdravih adolescenata (bez psihijatrijske dijagnoze). U istraživanju su primenjeni sledeći instrumenti: opšti Socio-demografski upitnik, Strukturisani klinički intervju (SCID-I) za Dijagnostički i statistički priručnik za duševne poremećaje – IV izdanje (DSM-IV), Bekov upitnik za procenu depresivnosti, Inventar afektivnog vezivanja prema roditeljima i vršnjacima i Upitnik o emocionalnoj regulaciji. Međugrupne razlike analizirane su MANCOVA metodom, a povezanost parcijalnom korelacijom, uz pol, starost i red rođenja kao kovarijante. **Rezultati.** U grupi depresivnih adolescenata, afektivno vezivanje za majku i za vršnjake bilo je nesigurnije nego kod zdravih adolescenata, a vezivanje za oca nesigurnije u odnosu na ostale dve grupe (MANCOVA F = 4,571; $p = 0,000$). Adolescenti sa dijagnozom anksioznog poremećaja imali su nesigurnije afektivno vezivanje za oca i za vršnjake u odnosu na adolescente bez psihijatrijske dijagnoze ($p < 0,05$). Depresivni adolescenti ređe su koristili veštinu kognitivnog promišljanja u odnosu na obe kontrolne grupe (MANCOVA F = 5,200, $p = 0,001$). Subjektivni doživljaj depresivnih simptoma bio je povezan sa nesigurnim afektivnim vezivanjem za oba roditelja i vršnjake (r = -0,457; -0,436, -0.349 respektivni, $p = 0,000$) kao i sa slabijom upotrebom kognitiv-

Correspondence to: Željka Košutić, Institute of Mental Health, 11 000 Belgrade, Serbia.

nog promišljanja (r = -0,446; p = 0,000). **Zaključak.** Naši rezultati upućuju na povezanost nesigurnog afektivnog vezivanja u svim domenima nesigurnog afektivnog vezivanja i depresije u adolescentnom periodu, uz specifičnu slabost emocionalne regulacije (slabo kognitivno promišljanje). Dobijeni nalazi mogu imati praktične implikacije za kreiranje preventivnih i terapijskih intervencija.

Ključne reči:
adolescenti; depresija; emocije; afektivno vezivanje.

Introduction

Depression among adolescents represents an important and controversial phenomenon in psychiatry, having in mind its high prevalence, serious consequences and difficulties in diagnostic process [1]. Studies show that as much as 20% of adolescents at the end of this developmental period have a high life prevalence of depression [2]. Depression of early age may have a more severe form than the one which occurs later [3], as it may lead to serious long-term dysfunction and suicide [3–6]. Still, there is a lack of knowledge about it, due to the impact of developmental variations in its manifestations which can be an obstacle in adequate recognition and treatment [1].

One of the factors associated with the development of depression is emotion regulation, a set of processes involved in modification of dynamics and intensity of emotional experience [6]. The emotion regulation skills are created in childhood, show individual stability and can play an important role in the adjustment processes [7]. The emotion regulation skills can be divided into two basic strategies: cognitive reappraisal strategy, which reframes the meaning of situation and effectively regulates further genesis of emotional experience and behavior, and expressive suppression strategy adopted after the genesis of emotions and leads to reduced expression of emotional behavior, with minimal impact on the actual emotional experience [8]. It was shown that more frequent use of cognitive reappraisal strategy is related to good adaptive outcomes, while more frequent use of expressive suppression in a longer period of time may be related to depressive symptoms [9]. It was also shown that maladaptive emotion regulation is associated with suicidality in children and adolescents, even with the control of depressive disorder [8]. Another factor, important for the mental health of adolescents, refers to the attachment to parents, as well as to peers – important attachment figures in this developmental period [10]. Patterns of attachment to parents represent internal working model for further interpersonal functioning [11]. Secure attachment style represents the characteristic of good adaptability, while insecure attachment style plays an important role in the development of depressogenic models of adaptation and is a risk factor for the emergence of depressive disorders [10]. It was shown that insecure attachment to parents and peers is a risk factor for depression [12], and that insecure attachment to parents could be significantly associated with the suicidality in adolescent population [13].

In addition to depression, the attachment patterns and emotion regulation strategies may be closely related to other internalizing psychiatric disorders in adolescence, such as anxiety disorders. Anxiety disorders represent the most frequent mental disorder of adolescence [14], and may contribute to 2–3 fold increase in risk of having both anxiety and depressive didorders in adulthood [15]. Similarly to those with depression, it was shown that adolescents with anxiety disorders have difficulties in emotion regulation as well [16]. On the other hand, numerous studies that explored the effect of attachment on mental health, in the last ten years, point out to the importance of this link not only in depression, but also in relation to anxiety symptoms within anxiety and depressive disorders [17].

Although previous studies suggest a correlation between the above stated factors with the intensity of depressive symptoms among adolescents, the data on these relationships among adolescents with clinically manifested depressive disorder are scarce. Also, there is insufficient data on the specifics of these phenomena in depression comparing not only to healthy subjects but also to anxiety disorders in adolescence as well.

This study was aimed to explore the attachment and emotion regulation skills among adolescents with depressive disorders.

Methods

This cross-sectional study involved 101 participants from the area of Belgrade and surroundings; 52 (50.5%) were female and 49 (49.5%) male participants, age 16 to 24 years (mean age 18.95 ± 2.23 years). The sample consisted of three groups. Two patient groups were recruited consecutively, whereas healthy controls were engaged as a convenience sample, during the period January to October 2014.

The first group involved 41 patients of the Day Hospital for Adolescents at the Institute of Mental Health that fulfilled criteria for depressive disorder according to the Diagnostic and Statistical Manual of Mental Disorders – (DSM-IV) classification of mental disorders [18]. The second group involved 30 patients from the same institution, who had fulfilled the DSM-IV criteria for anxiety disorders. Both groups of patients were tested in the initial phase of treatment in the Day Hospital for Adolescents. The third group involved 30 high-school students from urban part of Belgrade who were never psychiatrically treated (healthy controls).

The exclusion criteria from the study were persons with previous or current psychotic symptoms, bipolar disorder, intellectual disability and substance abuse.

Among these three groups no statistical differences in gender was found (χ^2 = 0.469; p = 0.791) and place of living (all of them were from Belgrade and surroundings). The healthy adolescents were younger than participants with anxiety disorder (ANOVA F = 5.319; p = 0.006; the Turkey HSD test: mean difference 1.8 years, p = 0.004), and had the later birth order comparing to both clinical groups (ANOVA

F = 6.231; $p = 0.003$; the study group – the Turkey HSD test: mean difference 0.519; p = 0.004; the psychiatric control group – the Turkey HSD test: mean difference 0.467; p = 0.018). These differences in age and birth order among the groups statistically (as covariates in multivariate analyses) were controlled. All participants signed informed consent (with parental permission for minors) at the beginning of the study. The study was approved by the Ethics Committee of the Institute of Mental Health, and was conducted according to the good research practice guidance provided of the School of Medicine, University of Belgrade.

Instruments

The participants were assessed by the self-report and interview instruments.

The socio-demographic questionnaire was developed for the purpose of this research. It explored the socio-demographic characteristics of adolescents as well as the data about previous psychological difficulties and psychiatric treatment.

The structured Clinical Interview for DSM-IV disorders, (SCID-I) was used [18]. The depressive disorders included were presented as major depression (depressive episode, recurrent depressive disorder), dysthymic and other depressive disorders. The anxiety disorders involved generalized anxiety disorder, panic disorder, panic disorder with agoraphobia, agoraphobia without history of panic disorder, specific phobia, social phobia, obsessive compulsive disorder, posttraumatic stress disorder and acute stress disorder.

The Beck Depression Inventory (BDI) was also used as well as the Emotional Regulation Questionnaire (ERQ).

BDI [19] is a questionnaire measuring severity of depressive symptoms, through 21 items with 4-point scale answers. The final depression score is a sum of scores on each item.

ERQ [20] is a questionnaire in which participants report personal skills in emotion regulation: cognitive reappraisal and expressive suppression. These two scales are presented as continuous variables, through 10 items on a 7-point Likert-type scale.

The Inventory of Parent and Peer Attachment (IPPA) [21] measures the level of attachment of adolescents to mother, father and peers. Modalities are expressed through separate scales, which consist of 25 items each, with 5-point scale answers. The scores are sums of relevant items, with reverse coding of some items, and with a higher value scores indicating more secure attachment.

Statistical analysis

Data analysis was carried out by using the IBM Statistical Package for the Social Sciences (SPSS) software, version 20.0. The data were processed using *t*-test, multivariate analysis of covariance (MANCOVA) and partial correlation. In multivariate analyses, gender, age and birth order were used as coviariates due to their significant relationship with main variables. Differences were considered statistically significant when the *p*-value was < 0.05.

Results

Gender, age, and birth order were associated with main variables in different ways. Female participants had more secure attachment to peers comparing to males ($t = -2.628$; $p = 0.01$; mean difference 9.44), while males used expression suppression more, with marginal significance ($t = 1.937$; $p = 0.05$; mean difference 1.99). The older adolescents had less secure attachment to father (r = -0.263; $p = 0.009$). Those with later birth order had more secure attachment to father (r = 0.286; $p = 0.004$) and peers (r = 0.274; $p = 0.006$), whereas the association between birth order and attachment to mother was marginally significant (r = 0.194; $p = 0.053$).

The attachment differences between groups were statistically significant, controlling for gender, age and birth order (MANCOVA: Wilk's lambda = 0.753, F = 4.571; $p = 0.000$) in all three domains (Table 1).

Post-hoc contrast results (Table 2) showed less secure attachment to mother and peers in the depressed patients comparing to the healthy controls, while their attachment to father was less secure comparing to both control groups. The adolescents with anxiety disorders had less secure attachment to father and peers in comparison with the healthy controls.

The emotion regulation differences among the groups, controlling for gender, age and birth order were statistically significant as well, (MANCOVA: Wilk's lambda = 0.811, F = 5.200; $p = 0.001$), but only in the domain of the cognitive reappraisal, whereas no significant differences were found in expressive suppression (Table 3).

Table 1

Attachment differences among groups, controlling for gender, age and birth order

Attachment type	Depressed adolescents (mean ± SD)	Anxiety controls (mean ± SD)	Healthy controls (mean ± SD)	Between-subjects effects (MANCOVA)
Attachment to mother	83.4 ± 20.99	92.60 ± 19.20	98.60 ± 17.22	F = 3.167, $p = 0.047$
Attachment to father	70.12 ± 20.48	80.90 ± 27.68	99.77 ± 16.01	F = 10.518, $p = 0.000$
Attachment to peers	90.24 ± 16.84	88.37 ± 20.25	104.03 ± 15.25	F = 4.415, $p = 0.015$

SD – standard deviation; MANCOVA – multivairate analysis of covariance.

Table 2

Post-hoc group-by-group comparisons for attachment and emotion regulation

Posthoc	Attachment to mother	Attachment to father	Attachment to peers	Cognitive reappraisal
MANCOVA contrast significance	DA/AC	DA /AC*	DA /AC	DA /AC**
	DA /HC*	DA /HC***	DA /HC*	DA /HC***
	AC/HC	AC/HC*	AC/HC**	AC/HC

DA – Depressed adolescents; AC – Anxiety controls; HC – Healthy controls; MANCOVA – multivariate analysis of covariance; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3

Emotion regulation differences between groups, controlling for gender, age and birth order

Emotion regulation strategy	Depressed adolescents mean ± SD	Anxiety controls mean ± SD	Healthy controls mean ± SD	Between-subjects effects (MANCOVA) F, *p*
Cognitive reappraisal	22.00 ± 9.07	28.97 ± 8.74	30.07 ± 6.44	F = 9.579, $p = 0.000$
Expression suppression	15.09 ± 5.62	14.90 ± 4.85	13.17 ± 4.97	F = 1.338, $p = 0.267$

SD – standard deviation; MANCOVA – multivairate analysis of covariance.

Post-hoc contrast results (Table 2) showed that the adolescents with depressive disorders used the cognitive reappraisal less, comparing to both control groups.

Controlling for gender, age and birth order, scores on BDI in the total sample were inversely partially correlated with attachment to mother (r = -0.457; $p = 0.000$), father (r = -0.436; $p = 0.000$) and peers (r = -0.349; $p = 0.000$) as well as with cognitive reappraisal (r = -0.446; $p = 0.000$), whereas there was no significant association with expressive suppression (r = 0.124; $p = 0.224$).

Discussion

Our findings showed the insecure attachment patterns in the adolescents with clinical manifestation of depressive disorders, comparing to the healthy controls. These results, as expected, confirm the findings of correlation between insecure attachment and depression in previous studies of adolescents [22–26] as well as in studies of adults [10]. Small number of studies dealt with depressive disorder among adolescents [22]. Apart from major depression disorder, previous research rarely included other depressive disorders, which was the case in our study. Furthermore, small number of studies separately explored attachment to mother, father and peers in this context [23]. In our study, the depressed participants, comparing to adolescents from the healthy population, had less secure attachments to both parents as well as to peers, and the subjective experience of depressive symptoms in our sample was significantly correlated with all three domains of attachment. When the attachments to both parents is considered, our findings are in accordance with the data from literature [22, 24]. Parents have the strongest social impact on children, and the attachment to them is one of the aspects of this crucial relationship, that, as a goal brings the sense of security, safety, protection, and represents the foundation for mental health [22]. Agerup et al. [22], however, noticed that previous studies most frequently explored the attachments to both parents together, or only to mothers, and stress the importance of analyzing the attachment to mother and father separately.

In our study, the depressed participants had insecure attachment both to mother and father comparing to the healthy controls, whereas in comparison with the adolescents with anxiety disorders, the attachment insecurity of the depressed patients was detected only in relation to father. These findings speak in favor of the previous findings of inverse correlation between mother's/father's support and depressive symptoms among adolescents [27]. In the study done by Van Roekel et al. [27], however, the insufficient support by mother was related to depressive symptoms only for girls, while the weak support of father was related with depression only for boys. In our study, insecure attachment both to mother and father among depressed participants was independent of gender, age and birth order.

The relationship of depression and insecure attachment to mother is in accordance with a well-known fact that the early loss of mother is a risk factor for depression as well as that the problems in early relationship with mother represent the risk factor for depression in adulthood [28]. The need to study attachment to father's figure in the context of the maladaptive outcomes for children, is increasingly recognized in literature [29]. The attachment to father in our sample was the least secure in the adolescents with depression, more secure in those with anxiety disorders, and the most secure in the group of healthy controls. Such results may point out to the possibly specific role of attachment to father in adolescent depression comparing to anxiety disorders. As at the other side of the mirror, conflicing father-child relationship in previous research, was shown as a close association to depression in fathers, that, on the other hand, was found to be significantly related to child's internalized and externalized psychopathology [29].

When it comes to attachment to peers, the data in literature is scarce and inconsistent. Some studies presented a relationship of insecure attachment to peers and depression among adolescents [23, 30, 31] while other did not show this association [22]. Adolescents with secure attachment to peers were characterized by capacity to form close relationships with peers while maintaining autonomy [31]. The formation of

adequate relationships to peers is important for self-confidence, however, when it is violated, the road to adolescent depression is open [32]. The good relationships with peers are necessary for the development of healthy personality in adolescence and can also decrease the negative influence of inadequate attachment to parents on personality development [33].

It is known that insecure attachment could be of importance not only for the development of depression but also for the wider spectrum of psychopatological manifestations [18], such as anxiety disorders [34, 35], tendencies towards substance abuse, conduct disorders, and personality disorders [36]. Whereas some studies showed contributions of attachment to parents to both depression and anxiety, with different pathways hypothesized [37], data on relative roles of attachment to mother and father in depression vs. anxiety are still insufficient. The participants with anxiety disorder in our study had less secure attachment to father and peers compared to the healthy controls and more secure attachment to father compared to the depressive participants. Their mean scores of attachment to mother was somewhere between the scores of the depressed and healthy controls, but the differences were not statistically significant. These findings may imply that parent, more specifically father-attachment security among anxious patients in our study, falls somewhere between the depressive and healthy group, and consequently, that attachment security may not only show qualitatively different trajectories to depression vs. anxiety, but that these differences may be also quantitative. In line with that, if internalizing disorders were represented on a gradual continuum, depression could be perceived as a more severe form of attachment disturbance than anxiety disorders.

When emotion regulation was considered in our study, subjects with depressive disorder had less adaptive emotion regulation comparing to both subjects with the anxiety disorder and healthy controls. The stronger subjective experience of depressive symptoms among our participants was also correlated with weaker emotion regulation. This finding is in accordance with previous studies which showed that dysfunctional emotion regulation was a significant factor that not only increased the risk for the development of depression [38] but also manifested after the recovery from depression, and increased the risk for depression relapse [39]. Emotion dysregulation of our depressed adolescents manifested through less frequent use of cognitive reappraisal, comparing to both subjects with the anxiety disorders and healthy controls.

The aforementioned findings could point out to the specific problems of emotion regulation in depression that could precisely be related to insufficient cognitive reevaluation of emotional stimuli. This is in accordance with previous findings of depressed persons using cognitive reappraisal less than healthy subjects [38–42]. Our results are in accordance with the hypothesis on cognitive reappraisal being the protective factor, therefore the one which could prevent the development of depression [38]. However, when suppression of expression is considered, our results are not in accordance with the previous studies that recognized it as a maladaptive emotion regulation strategy, since it was more used by persons with depression [38–42]. Our study did not show significant differences in expressive suppression among the groups nor was there a significant correlation of this strategy with subjective experience of depressive symptoms in our sample. One of the reasons for this finding could refer to the fact that adolescents who are currently in psychiatric treatment are motivated, compliant and with better insight, thus less prone to suppressing emotional expressions. Even though the literature showed that weaker emotional regulation is also present among other mental disorders, not only in depression [33], subjects with anxiety disorders in our study did not show significant differences in this domain, comparing to the healthy adolescents. These results are in the contrast to previous studies that found more dysfunctional emotion regulation in adolescents with anxiety disorders, comparing to healthy ones [43]. It was shown that anxious adolescents use adaptive emotional regulation strategies less than non-clinical subjects [44], i.e., the cognitive reappraisal, while they use more the expressive supression as the maladaptive model of emotional regulation [45]. In our study, however, the emotional regulation of anxious adolescents was closer to the one of the healthy subjects than it was to the depressive ones, which may, as well as when it comes to attachment, point out to possible specific patterns in the adolescent depression.

Our study has several limitations. The first one refers to the cross-sectional study design which makes it impossible to make conclusions about causal relationships. Secondly, the number of subjects in the groups was not high, which could impact the statistical significance and lead to underestimation of significant relationships. Finally, the participants with depression and anxiety disorders in the study were currently involved in psychiatric treatment that could influence the obtained data. However, our study has certain advantages. To our knowledge, it is one of the rare studies that examined clinically manifested adolescent depressive disorders in the context of attachment and emotion regulation while making a differentiation in attachment to mother, father and peers. It was, as well, one of the rare studies to compare the depressed adolescents not only the healthy controls, but also to compare the adolescents with anxiety disorders, with the control of gender, age and birth order.

Conclusion

The results of our study showed the relationship of insecure attachment in all domains with adolescent depression. This relationship refers both to depression and subjective experience of depressive symptoms. The adolescent depression in our study was specifically associated with emotional dysregulation through less frequent use of cognitive reappraisal, thus weaker reevaluation of emotional stimuli independently of gender, age and birth order in family. Such findings may be of importance for planning the therapeutic interventions focused on the attachment and empowerment of cognitive reappraisal as an emotional regulation skill in interpersonal relationships, for adolescents with depressive disorder.

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Diagnostic value of noninvasive comprehensive morphological and functional assessment of coronary artery disease

Dijagnostička vrednost sveobuhvatne neinvazivne procene morfoloških i funkcionalnih karakteristika koronarne bolesti

Zorica Mladenović*†, Ana Djordjević-Dikić‡, Predrag Djurić*†, Andjelka Angelkov Ristić†§, Boris Džudović*, Zoran Jović*†

Military Medical Academy, *Clinic for Cardiology, §Clinic for Emergency Internal Medicine, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, Clinical Centre of Serbia, ‡Institute for Cardiovascular Diseases, Belgrade, Serbia

Abstract

Background/Aim. Recently adopted technique, Transthoracic Doppler Echocardiography (TDE) enables the estimate of functional significance of coronary luminal narrowing. A multi-slice computed coronary angiography (MSCT), as one of the most important noninvasive methods, offers us a quite precise visualization of morphological characteristics of atherosclerotic changes in coronary arteries. We have tried to evaluate the most reliable noninvasive approach aimed at the detection of major stenosis on the left anterior descending artery (LAD) and the right coronary artery (RCA). **Methods.** This study involved 84 patients, with the previously detected atherosclerotic lesions on the LAD and/or RCA by MSCT. The coronary flow reserve (CFR) assessment by TDE with adenosine was obtained in LAD (n = 75); RCA (n = 61), resulting in 136 vessels subjected to the analysis. Invasive coronary angiography (ICA) was performed in all patients within 24 to 48 hours after the CFR as a reference technique. **Results.** The Cochrans Q test proved a significant statistical difference among these techniques in detection of a significant stenosis on the LAD and RCA ($p < 0.01$). Further analyses revealed a significant difference between the MSCT and CFR ($p < 0.05$), MSCT and ICA ($p < 0.01$), whereas we did not find a significant difference between the CFR and ICA ($p > 0.05$). The main discrepancies in results among the CFR, ICA and MSCT were noticed concerning intermediate and severe stenosis on the MSCT. The MSCT had a diagnostic accuracy for the LAD 66.67%, for the RCA 75.%, the CFR had for the LAD 90% and for the RCA 81.67%, in detection of significant stenosis. Where the consensus was reached between both techniques, diagnostic accuracy was improved for the LAD 97.33% and the RCA 90 %. **Conclusion.** Comprehensive noninvasive evaluation of both anatomical and functional imaging in coronary diseases makes the optimal approach for precise, noninvasive assessment of the coronary artery lesions in the coronary arteries.

Key words:
coronary artery disease; diagnosis, differential; blood flow velocity; echocardiography, doppler; multidetector computed tomography; coronary angiography; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Nedavno usvojena tehnika, Transtorakalna Doppler Ehokardiografija (TDE) omogućava procenu funkcionalne značajnosti suženja koronarne arterije. Koronarografija putem "multi-slice" kompjuterizovane tomografije (MSCT) nam pruža informacije o morfološkim karakteristikama koronarne arterijske bolesti. Cilj istraživanja je bio da se proceni najpouzdaniji neinvazivni dijagnostički pristup u cilju detekcije značajnih stenoza na prednjoj descedentnoj arteriji (LAD) i desnoj koronarnoj arteriji (RCA). **Metode.** Studijom je obuhvaćeno 84 bolesnika, sa prethodno detektovanim aterosklerotskim lezijama na LAD i/ili RCA putem MSCT. Procena koronarne rezerve protoka (CFR) putem TDE sa adenozinom sprovedena je na LAD (n = 75) i RCA (n = 61), sa ukupno 136 koronarnih arterija za analizu. Invazivna koronarografija (ICA) je urađena kod svih bolesnika 24 do 48 sati posle CFR. **Rezultati.** Cochrans Q testom je dokazana je statistički značajna razlika između tehnika pri detekciji značajnih koronarnih lezija na LAD i RCA ($p < 0,01$). Dalje analize su ukazale na značajnu razliku između MSCT i CFR ($p < 0,05$), MSCT i ICA ($p < 0,01$), dok između CFR i ICA nije uočena statistički značajna razlika ($p > 0,05$). Najveća diskrepanca u rezultatima uočena je između CFR, ICA i MSCT kada su analizirane interemdijarne teške stenoze. Dijagnostičku

Correspondence to: Zorica T. Mladenović, Vojislava Ilića 22, Belgrade 11 000, Serbia. E-mail: zoz3377@gmail.com

pouzdanost MSCT za LAD je bila 66,67%, za RCA 75 %, a CFR za LAD 90% i za RCA 81,67%, pri detekciji značajnih aterosklerotskih lezija. Kada su rezultati obe tehnike bili u saglasnosti dijagnostička pouzdanost je unapređena i za LAD (97,33%) i za RCA (90%). **Zaključak.** Sveobuhvatna neinvazivna procena, kako morfoloških, tako i funkcionalnih karakteristika koronarne bolesti je optimalan pristup za neinvazivnu i preciznu procenu značajnosti aterosklerotskih lezija na koronarnim arterijama.

Ključne reči:
koronarna bolest; dijagnoza, diferencijalna; krv, brzina protoka; ehokardiografija, dopler; tomografija, kompjuterizovana multidetektorska; angiografija koronarnih arterija; senzitivnost i specifičnost.

Introduction

A multislice computed tomographic (MSCT) coronary angiography is frequently used as a noninvasive diagnostic procedure for evaluating calcified and non-calcified atherosclerotic lesions and their diameter of coronary artery [1–3]. The MSCT has a very high negative predictive value while it is less reliable in assessing a real physiological significance of the coronary stenosis [4–9]. Consequently, a noninvasive multimodality imaging strategy which could provide to us morphological and functional information was appreciated. The purpose of this study was to define the importance of coronary flow reserve (CFR) determined by Transthoracic color Doppler Echocardiography (TDE) over MSCT in detection of the hemodynamically significant stenosis on the left anterior descending artery (LAD) and the right coronary artery (RCA). The reference technique was the Invasive coronary angiography (ICA).

Methods

This prospective study included 84 patients (mean age 61.79 ± 9.21 years). The ICA was recommended due to formerly ambulatory detected atherosclerotic lesions on the coronary arteries (LAD or/and RCA) by the MSCT angiography. The MSCT was preformed because of the previous inconclusive noninvasive tests. The additional measurements of the CFR by the TDE were performed on 136 coronary arteries 24–48 hours before the ICA.

The exclusion criteria for the MSCT were pregnancy, renal failure, atrial fibrillation, and frequent extra systolic beats. While the exclusion criteria for the CFR test were a high degree atrioventricular block, acute myocardial infarction, unstable angina, a significant myocardial hypertrophy, an obstructive pulmonary disease or a previous therapy with theophylline preparations. The subjects excluded xanthine-containing food and drinks for at least 24 hours before the test. The study protocol was submitted and certified by the medical ethical committee of the hospital. and all patients gave informed consent.

MSCT coronary angiography

The 64-slice scanner (Toshiba, Aquilion) with a 0.33 s rotation time was used for all CT scans. A 80 mL Iodixanol (Visipaque 320 mg/mL, Amersham Health, UK) was injected into an antecubital vein with a flow rate 5 mL/s and after that 50 mL saline bolus. An initial delay was determined by a bolus tracing in the descending aorta after which the Scan start was initiated 5 s after getting the threshold, (140 Hounsfield units – HU. Subsequently, scanning was done from the tracheal bifurcation to the diaphragm using: X-ray tube potential 120 kV, effective tube current 400 mA, slice collimation 64 mm^2, table feed 9.2 mm/rotation and pitch 0.24. Automated real time anatomy based dose regulation CARE Dose 4D was used during all CT scans. The overall scan time was less than 20. The average total time for the examination was 15 min. We used retrospective electrocardiographic (ECG) gating for the optimal heart phase selection and applied adaptive cardio volume approach for the data reconstruction. Throughout the examination, the axial slices were reconstructed by having been synchronized with the electrocardiogram (ECG) by a single or two sector algorithm (65 beats per minute – b.p.m) using data from consecutive heart beats. The pictures were reconstructed in 10% intervals of cardiac cycle in order to examine coronaries at that cardiac phase with almost minimal motion. The evaluation of coronary arteries was done with 0.5 mm thick slices and with a medium soft-tissue reconstruction kernel (FL03).

The coronary segments were defined pursuant to the American Heart Association (AHA) scheme [10] and the examination was undertaken by two independent observers unconscious of the former clinical history. First, both of them independently assessed the quality of visualization of each coronary segment and the presence of a hemodynamically significant stenosis, considered as luminal diameter reduction more than 70%. For any difference in data investigation, consensus agreement was reached.

Two-dimensional echocardiography – CFR measurement

The acoustic window was around the midclavicular line in the fourth or fifth intercostals space in the left lateral decubital position. In our echocardiographic study we used Vivid 7 (3.5 MHz and 7 MHz probes) and Doppler echocardiography for the evualtion of CFR in the distal portion of the LAD or RCA artery. In color Doppler flow mapping, the velocity range was set from 12.0 to16.0 cm/s to obtain the optimal imaging.

The coronary blood flow in the distal portion of the LAD was visualized by color Doppler, in the long axis cross-section of the left ventricle and in the anterior interventricular groove. The coronary flow velocity in the distal RCA was obtained from the posterior descending coronary artery (PD), in modified apical two-chamber view including the posterior interventricular groove.

Pulsed wave Doppler was used for the coronary flow velocity measurements. An angle correction was needed in each examination because of the incident Doppler angle (mean angle 28°, range 15–44°). The stop frames and clips were digitally documented. The coronary blood flow velocity profiles at the distal part of the coronary arteries were biphasic and it was estimated separately for the distal part of the LAD or posterior descending (PD) artery at baseline and after administration of adenosine (140 mcg/kg/min, lasting for 2 min). The CFR was calculated dividing the maximum hyperemic and resting peak diastolic flow velocity, so it is a non-dimensional parameter.

The average value of three cardiac cycles was used for the CFR measurement at basal and hyperemic conditions. A value of the CFR less than 2 was used to categorize a significant stenosis. All patients had un interrupted heart rate, blood pressure and the ECG monitoring.

Invasive coronary angiography (ICA)

The ICA was considered as the reference diagnostic technique. It was completed according to the standardized protocols and the images were recorded for the additional analysis. Two experts, familiar with the patient's clinical history, but unaware of the MSCT results, assessed independently all angiograms according to the same AHA scheme [3]. A significant coronary artery stenosis was defined as ≥ 70% diameter reduction. In case of any dispute over data analysis, consensus agreement was appended.

Statistical analyses

Data analysis was performed by the renowned statistical analysis software (SPSS 11.5, Chicago, Illinois). Statistically significant was a p value < 0.05. The Cochran's Q test and McNemar's test were used to assess differences concerning findings of the MSCT coronary angiography, CFR and ICA. Sensitivity, specificity, positive and negative predictive values of the MSCT and CFR were counted in the standard method. The Pearson's correlation coefficient was used to evaluate the relation of the MSCT, invasive angiographic and echocardiographic parameters.

Results

This prospective study analyzed 84 patients. Their main clinical characteristics are listed in Table 1. We also included patients who were previously revascularised with the percutaneous coronary interventions, 15/84 (17.86%) and coronary surgery, 3/84 (3.6%).

Table 1

Clinical characteristics of the patients (n = 84)

Characteristics of patients	Values
Gender, n (%)	
male	59 (70.2)
female	25 (29.8)
Age (years), mean ± SD	61.8 ± 9.2
Hypertension, n (%)	75 (89.3)
Family history for CAD, n (%)	49 (58.3)
Hyperlipidemy, n (%)	71 (84.5)
Diabetes mellitus, n (%)	25 (29.8)
Smoking, n (%)	32 (38.1)
Stress, n (%)	31 (36.9)
Ejection fraction (%), mean ± SD	59.01 ± 6.00
Wall motion score index, mean ± SD	04 ± 0.09
Collaterals, n (%)	23(27.4)
Number of coronary arteries with obstructive lesions, n (%)	
0	37 (44.0)
1	28 (33.3)
2	13 (15.5)
3	6 (7.1)

CAD – coronary artery disase; SD – standard deviation.

The CFR measurements were successfully obtained in the LAD (n = 75) and the RCA (n = 61), resulting in 136 vessels for analysis. Feasibility for the LAD was (75/77) 97.40% and for the RCA (61/70) 87.14% due to poor acoustic window.

We have presented findings of all diagnostic procedures including the invasive coronary angiography as a reference diagnostic technique in Table 2.

Table 2

Results of the multislice computed tomography (MSCT) and the coronary flow reserve (CFR) in relation to the invasive coronary angiography (ICA) findings

Coronary artery	MSCT (% of stenosis)			CFR	
stenosis (%)	< 50	50–70	≥ 70	> 2	< 2
LAD (n = 75)					
< 50	13	10	20	40	3
50–70	1	1	5	5	2
≥ 70	2	1	22	2	23
RCA (n = 61)					
< 50	11	3	15	25	4
50–70	1	3	2	4	2
≥ 70	2	1	23	6	20

LAD – anterior descending artery; RCA – right coronary artery.

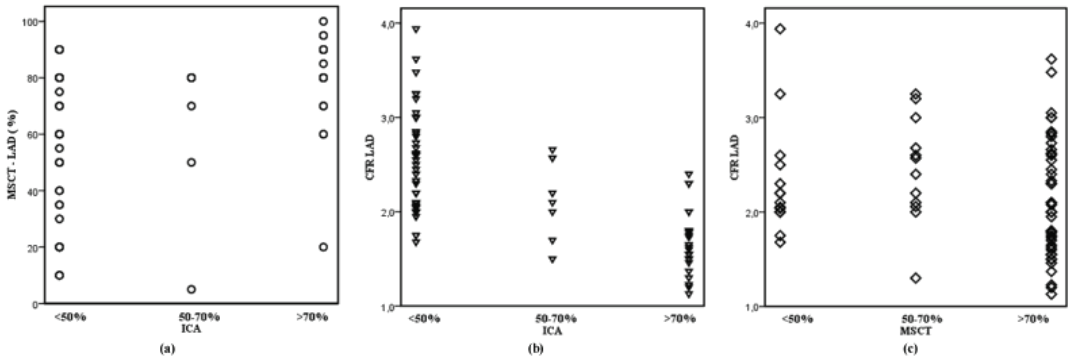

Fig. 1 – Scatterplots of the multislice computed tomography (MSCT) values in the obsreved left anterior descending (LAD) arteries with up to 50%, 50 to 70%, and over 70% diameter narrowing on the invasive coronary angiography (ICA) (a), coronary flow reserve (CFR) values in the observed LAD arteries with up to 50%, 50 to 70%, and over 70% diameter narrowing on the ICA (b) and the MSCT (c). Regarding detection of significant stenosis (> 70%) on the LAD there were a significant difference between the MSCT and the CFR ($p < 0.01$), the MSCT and the ICA ($p < 0.01$), while between the CFR and the ICA we did not find a significant difference ($p = 0.45$).

Difference between diagnostic techniques in the evaluation of the atherosclerotic lesions

A significant statistical difference, by the Cochrans Q test was found among these three techniques in detection of a significant lesion on the LAD (Q = 27.55, $p < 0.01$) and the RCA (Q = 8.96, $p < 0.01$). Additionally, further analyses revealed a significant difference between the results of the MSCT and the CFR (LAD: $p < 0.01$; RCA: $p < 0.05$), the MSCT and the ICA (LAD : $p < 0.01$; RCA: $p < 0.01$), while between the CFR and ICA we did not find a significant difference (LAD: $p = 0.45$; RCA: $p = 1.00$).

Diagnostic techniques in detection of the atherosclerotic lesions on LAD

A significant atherosclerotic coronary artery stenosis (≥ 70%) on the LAD by the ICA as reference method was detected in 25 (33.33%) of 75 arteries, the lesions of intermediate diameter (50–70%) were present in 7 (9.33%) of 75 and in 43 of 75 (57.33%) The ICA detected lesions less than 50% of diameter reduction.

The MSCT verified 47 (62.70%) obstructive lesions in 75 observed LAD arteries, 22 (46.81%) of 47 significant lesions on the MSCT were in agreement with the ICA while 25 (53.11%) of 47 obstructive lesions on the MSCT were overestimated comparing with the ICA (5 of them were the intermediate lesions and 20 non-significant lesions on the ICA). The MSCT find out 12 (16.00%) of 75 stenosis of the intermediate diameter (50–70%), but 10 (83.33%) of 12 on the ICA were the non-significant lesions. The MSCT detected 16 (21.33%) of 75 vessels with the non-significant lesions (< 50%); there was the agreement with the ICA in 13 (81.25%) of 16 segments, while only 3 (18. 75%) of 16 were underestimated. (Table 2, Figure 1a).

Comparing measurements of the coronary flow reserve and the MSCT angiography on the LAD, there was a significant difference ($p < 0.01$) (Table 3). We found that the CFR results agreed in detection of the significant lesions with the MSCT in 24 (51.06%) of 47 vessels, while in 23 (48.94%) of 47 arteries, the coronary flow reserve was preserved and excluded their functional significance. Concerning the intermediate lesions detected by the MSCT, the CFR found the preserved coronary flow reserve in 11 (91.67%) of 12 arteries with the intermediate stenosis that excluded their functional significance. Regarding normal vessels on the MSCT, there was the agreement with the CFR in 13 (81.25%) of 16 vessels, while 3 of them were underestimated with the CFR. (Table 2, Figure 1c).

The CFR measurements detected in 28 (37.33%) of 75 LAD arteries the hemodynamically significant (CFR < 2) stenosis and it was in concordance with the ICA in 23 (82.14%) of 28 vessels, while 5 (17.86%) of them had no significant atherosclerotic lesions on the ICA (2 were intermediate and 3 non-significant lesions on the ICA) and they were overestimated by the CFR. The CFR graded 47 (62.67%) of 75 LAD arteries as normal, and it was in concordance with the ICA in 40 (85.11%) of 47 arteries, but 7 of 47 were underestimated (5 of them were the intermediate lesions and 2 vessels had the obstructive lesions on the ICA) (Table 2, Figure 1b).

Diagnostic techniques in detection of atherosclerotic lesions on RCA

The ICA detected 26 (42. 62%) of 61 significant stenosis on the RCA, 6 (9.83%) of 61 were the intermediate lesions and 29 (47.54%) of 61 were the non-significant lesions.

The MSCT found 40 (65.57%) the obstructive atherosclerotic lesions of 61 RCA arteries and correctly identified 23 (57.50%) of 40 significant lesions, while 17 (42.50%) of 40 were overestimated comparing with the ICA (2 were intermediate lesions and 15 were non significant on ICA). Regarding lesions of intermediate range MSCT verified 7 (11.48%) of 61, but 3 (42.86%) of 7 were over estimated comparing with ICA, 3 (42.86%) of 7 were correctly classified as the intermediate stenosis, and 1 of 7 (14.29%) was underestimated. MSCT detected 14 (22.95%) of 61 stenosis less than 50% diameter and it was in agreement with the ICA in 11 (78.57%) of 14 cases while 3 (21.43%) of 14 were underestimated by the MSCT (Table 2, Figure 2a).

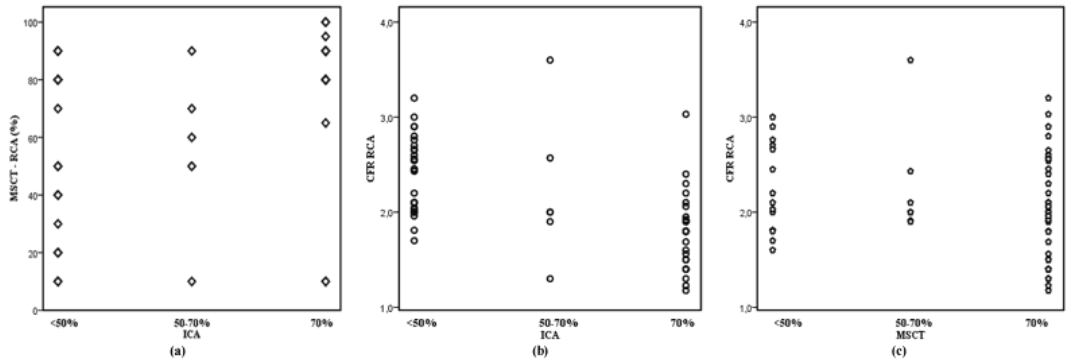

Fig. 2 – Scatterplots of the multislice computed tomography (MSCT) values in the observed right coronary arteries (RCA) with up to 50%, 50 to 70%, and over 70% diameter narrowing on the invasive coronary angiography (ICA) (a), coronary flow reserve (CFR) values in the observed left anterior descending (LAD) arteries with up to 50%, 50 to 70%, and over 70% diameter narrowing on the ICA (b) and the MSCT (c). Regarding detection of significant stenosis (> 70%) on the RCA there were a singificant difference between the MSCT and the CFR ($p < 0.05$), the MSCT and the ICA ($p < 0.01$), while between the CFR and the ICA we did not find a significant difference ($p = 1.00$).

Table 3

The multislice computed tomography (MSCT) versus the coronary flow reserve (CFR) results on the anterior descending artery/right coronary artery (LAD/RCA)

MSCT	LAD (n = 75)			RCA (n = 61)		
(% of stenosis)	CFR > 2	CFR < 2	Total	CFR > 2	CFR < 2	Total
< 50	13	3	16	10	4	14
50–70	11	1	12	5	2	7
≥ 70	23	24	47	20	20	40
Total	47	28	75	35	26	61

Table 4

The diagnostic value of the multislice computed tomography (MSCT) coronary angiography and the coronary flow reserve (CFR) detected by the transthoracic doppler echocardiography (TDE)

Parameters	LAD (n = 75			RCA (n = 61)		
	MSCT	CFR	MSCT & CFR	MSCT	CFR	MSCT & CFR
Sensitivity (%)	88.00	92.00	92.00	86.21	76.92	76.92
Specificity (%)	57.63	90.00	100.00	69.10	85.29	100.00
Positive predictive value (%)	46.81	82.14	100.00	59.52	80.00	100.00
Negative predictive value (%)	91.19	95.74	96.15	90.48	82.86	85.01
Diagnostic accuracy (%)	66.67	90.00	97.33	75.00	81.67	90.00

Comparing the measurements of the CFR and MSCT on the RCA there was a significant difference ($p < 0.05$) (Table 3). The CFR was in agreement with the MSCT in detection of the significant lesions in 20 (50%) of 40 vessels which were graded on the MSCT as a stenosis (over 70% diameter), while 20 (50%) of them had preserved the coronary flow reserve. Concerning the intermediate lesions on the MSCT, the CFR detected the preserved coronary flow reserve in 5 of 7 (71.43%) RCA arteries and excluded their real hemodynamic significance. Regarding the lesions up to 50% on the MSCT, there was an agreement between these techniques in 10 of 14 (71.43%), while 4 of them were the hemodinamically significant lesions by the CFR (Table 2, Figure 2c).

The CFR measurements by the TDE verified in 26 of 61 (42.62%) flow limiting stenosis on the RCA and they were in agreement with the ICA in 20 (76.92%) of 26 vessels, while 6 of them were overestimated comparing with the ICA (2 were the intermediate lesion and 4 non-significant). The CFR detected a normal coronary flow in 35 (57.38%) of 61 vessels and correctly identified 25 (71.43%) of 35 non-significant lesions. Although 10 (28.57%) of 35 were underestimated (4 of them were the lesions of the intermediate diameter and 6 were the obstructive lesions) (Table 2, Figure 2b).

Diagnostic significance of MSCT coronary angiography and CFR measurements findings by the TDE

The results of sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of the MSCT coronary angiography and the CFR are presented in Table 4. The MSCT had a high sensitivity, negative predictive value, while the CFR measurements presented the better values of specifity and positive predictive value. When the results of both methods were in concordance regarding importance of the atherosclerotic lesions on the LAD or on the RCA, it increased a diagnostic accuracy of noninvasive detection of the obstructive lesions, that indirectly imply the importance of both the morphological and functional assessment of the atherosclerotic lesions.

Discussion

Invasive coronary angiography still remains the "gold standard" for detection of a significant obstructive coronary disease, using the percentage of a diameter stenosis with the cut-off values of 50%, or 70%. In recent years, the MSCT coronary angiography has been increasingly used as a noninvasive imaging technique capable of detecting non-significant and obstructive atherosclerosis and exclude it with a high diagnostic certainty like its greatest attributes [1–3, 11].

Some previous comparative studies between the MSCT and functional tests (nuclear perfusion imagining) reported a good concordance between these modalities in the case of the normal MSCT. These observations are in line with the high negative predictive value observed comparing the MSCT with the ICA and indicate that patients with a normal MSCT do not need further evaluation [3, 11]. A significantly lower agreement was found between the anatomical and functional approaches in patients with the intermediate and significant atherosclerotic lesions on the MSCT. Usually, in case of significant lesions on the MSCT, patients had almost a normal perfusion scans [12–14]. Motion artifacts due to ventricular ectopic beats and blooming effects of calcifications decrease precise lumen visualization, which predisposes to overestimation of severity of the coronary atherosclerotic lesions by the MSCT and result in a low positive predicative value [14]. Regarding the coronary arteries with the non-significant atherosclerotic lesions, the results of our study revealed a high agreement between results of the invasive and multislice coronary angiography (LAD: 81.25%, RCA: 78.57%), also between results of the MSCT and a functional assessment of the atherosclerotic lesions by the CFR (LAD: 81.25%, RCA: 71.43%). We could notice to some extent an inferior concordance of results between the techniques regarding the RCA, due to its worse visualization because of a high mobility and smaller diameter of the RCA comparing with the LAD. These results are in line with previous reports and point out that a normal coronary anatomy by the MSCT could accurately exclude the presence of the hemodynamically significant CAD [15–17].

Accordingly, patients with minimal or non-obstructive atherosclerosis on the MSCT do not need any further evaluation and could be safely advised on medical treatment and reduction of risk factors for cardiovascular disease.

Several studies reported that the further functional evaluation following the MSCT was particularly needed in patients with intermediate, diffuse lesions on the MSCT, due to problems with defining real reference vessel diameters and low spatial resolution. In these groups of patients, the hemodynamic characteristics of an atherosclerotic lesion would determine whether an additional invasive diagnostic is indicated [15, 16, 18, 19]. Analyzing the intermediate stenosis detected by the MSCT, our investigation revealed a high proportion of the overestimated lesions in comparison with the ICA (LAD: 83.33%, RCA: 42.86%). In addition, the normal values of the CFR also excluded their real significance (LAD: 91.67%; RCA: 71.43%).

In case of a significant stenosis, our findings showed that the MSCT had, to some extent, a better agreement with the ICA (LAD: 46.81%; RCA: 57.50%) while still a high proportion of a significant stenosis on the MSCT were overestimated. A high proportion of these significant atherosclerotic lesions on the MSCT did not resulted in functional abnormalities on the CFR (agreement between MSCT and CFR for LAD: (51.06%; RCA: 50.00%) which indirectly implicated that the normal values of the coronary flow reserve could be used quite correctly to exclude the overestimated lesions on the MSCT coronary angiography.

Recent studies that compared the MSCT to the fractional flow reserve (FFR) [15, 16, 20] and investigations that assessed the MSCT to the CFR [17, 21] in prediction of a significant stenosis, reported that the significant lesions on the MSCT were not usually associated with the hemodynamically significant reduction of coronary flow reserve which indicated that the MSCT had a tendency to overestimate the degree of stenosis.

Previous studies that evaluated results of the MSCT and perfusion imaging [12–14] found a quite moderate relation between the MSCT and myocardial perfusion imaging. Indeed, the percentage stenosis was the only a moderate predictor of a perfusion defects. In evaluation of the severity of the coronary stenosis they did not include other factors that may modify myocardial perfusion, such as plaque morphology and endothelial function. Furthermore, diagnosis and therapy for patients with the suspected CAD [13, 22] will be a significantly improved if we take into account, at the same time, the results of perfusion imaging and the MSCT.

In our study, we also analyzed a diagnostic accuracy of the MSCT coronary angiography. Furthermore, we found a significant additive diagnostic role of a non-invasive measurement of the coronary flow reserve by the TDE, which significantly increased its diagnostic accuracy in recognition of a significant stenosis on the coronary arteries LAD and RCA (Table 4). This is the first study that analyzed the additive diagnostic value of the CFR over the MSCT results in the assessment of the atherosclerotic lesions on both arteries LAD and RCA. Previously, one study [21] presented similar results, which, in contrast to our investigation, compared the diagnostic value of the MSCT 40 slice scanner and the CFR estimating the atherosclerotic lesion on the LAD. There is also a comparable report of one small study preformed with the same technology, but concerning only the lesions on the LAD [17]. The additive diagnostic value of the CFR over the MSCT regarding the lesions on the RCA is not as good as with the lesions on the LAD, because of inferior feasibility, more complicated visualization due to high mobility and very often a small diameter of the coronary artery.

Transthoracic Doppler echocardiography with possibility to assess the Coronary Flow Reserve has been recognized as a reliable additive diagnostic tool in assessment of a functional significance of the coronary artery stenosis, but with limitations concerning microcirculatory dysfunction due to long- standing arterial hypertension, hyperlipidemy and diabetes mellitus that resulted in small percent of the abnormal CFR even in absence of the coronary stenosis [23]. The measurements of the TDE-CFR, in the LAD as in the PD arteries, are closely correlated with the invasive measurements using a Doppler flow wire [24–27].

The MSCT coronary angiography is reliable for excluding the presence of a significant coronary stenoses in patients with suspected coronary artery disease and this group of patients does not require any further invasive coronary angiography.

Nevertheless, the anatomical evaluation of coronary artery disease with the MSCT has its own limitations and an additional functional assessment is necessary, especially regarding a range of the intermediate and diffuse atherosclerotic lesions or obstructive stenosis. In everyday clinical work, the CFR could be useful additive diagnostic tool for the functional assessment of these atherosclerotic lesions, before the final decision on whether invasive evaluation is really necessary. Consequently, it would lessen a number of unnecessary invasive coronary angiographies and exposure to radiation.

Even though progress in the noninvasive evaluation of the coronary artery disease is obvious, we should also always think about its limitations, especially in patients with a significant left main stenosis or multivessel disease on the MSCT. It seems reasonable to refer these patients immediately to the catheterization laboratory for the additional invasive assessment in order to prevent any uncertainty.

Initially important lack of our study was a relatively small number of patients. Quantification of the coronary artery stenosis with the MSCT is still difficult in presence of extensive and diffuse calcifications, so the Ca score should be included in the final assessment of diagnostic value of the MSCT in order to improve its diagnostic parameters. The TDE-CFR measurements of a circumflexa (ACX) are unreliable for clinical practice. We used the invasive coronary angiography as a reference method but the Fractional flow reserve (FFR) would be more appropriate.

Conclusion

A comprehensive noninvasive anatomical and functional imaging could be the optimal way for a noninvasive assessment of the coronary artery lesions. This approach could most appropriately identify patients who should be safely referred for medical treatment and those who require an immediate invasive coronary angiography with a further revascularization.

Conflict of interest

This research received no grant from any funding agency in the public, commercial or not-for-profit sectors.

Authors declare that there is no conflict of interest.

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Analysis of knowledge and attitudes of the students of health and professional studies regarding the use of stimulative substances in sports

Analiza znanja i stavova studenata zdravstvenih studija o upotrebi stimulativnih supstanci u sportu

Goran Stojanović*, Milica Vasiljević Blagojević*, Zulfer Bahtijari†, Bratislav Stanković*, Dragana Terzić Marković*, Dušan Stojanović‡, Valentina Marinković§

***College of Health and Professional Studies in Belgrade, Belgrade, Serbia; †Pharmacy "Goodwill", Subotica, Serbia; ‡DeaMedica, Belgrade, Serbia; University in Belgrade, §Faculty of Pharmacy, Belgrade, Serbia;**

Abstract

Backrogund/Aim. Since the beginning of competitive sports, athletes have been trying to improve their abilities by taking various substances. The problem of using prohibited substances is not strictly tied to elite athletes; it is also present in the general population. The aim of this study was to test the knowledge and attitudes of the students regarding the use of stimulative substances and dietary supplements in sports. **Methods.** A cross-sectional study was performed among students at the College of Health and Professional Studies in Belgrade, Serbia. The data was collected by filling in an especially designed questionnaire. **Results.** Knowledge of prohibited substances and methods was characterized as "good" with 24.2% of respondents, namely 8.09% of males and 16.1% of females; knowledge of the adverse effects of prohibited substances and methods on health was demonstrated by 17.7% (9.03% of male respondents and 8.72% of female respondents). **Conclusion.** Student population is not knowledgeable enough about the problems of prohibited substances use and their negative effects on one's health. The comparative analysis of our and European researches on knowledge, attitudes and uses of prohibited substances show a rather uniform prevalence rate. Not being aware of the adverse effects shows the need to further educate students.

Key words:
doping in sports; students; dietary supplements; sterioids; knowledge; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Sportisti oduvek u takmičarskom sportu pokušavaju da poprave svoje sposobnosti uzimanjem različitih supstanci. Problem korišćenja nedozvoljenih supstanci nije vezan isključivo za elitne sportiste, već je prisutan i u opštoj populaciji. Cilj rada bio je ispitivanje znanja i stavova studenata u vezi sa upotrebom stimulativnih supstanci i dijetetskih suplemenata u sportu. **Metode.** Istraživanje je sprovedeno kao studija preseka kod studentske populacije u Visokoj zdravstvenoj školi strukovnih studija u Beogradu. Podaci su prikupljani popunjavanjem posebno dizajniranog upitnika. **Rezultati.** Dobro znanje o nedozvoljenim supstancama i metodama, pokazalo je 24,2% ispitanika (8,09% muškog pola i 16,1% ženskog pola), a o neželjenim efektima zabranjenih supstanci i metoda na zdravlje, znanje je pokazalo 17,7% ispitanika (9,03% muškog pola i 8,72% ženskog pola). **Zaključak.** Populacija studenata nema dovoljno znanja o problemu upotrebe nedozvoljenih supstanci i njihovim negativnim posledicama po zdravlje. Komparativna analiza našeg i evropskih istraživanja znanja, stavova i upotrebe nedozvoljenih supstanci ukazuje na približno ujednačene stope prevalencije. Nepoznavanje neželjenih efekata ukazuje na potrebu za dodatnom edukacijom studenata.

Ključne reči:
doping u sportu; studenti; ishrana, dopune; steroidi; znanje; ankete i upitnici.

Correspondence to: Goran Stojanović, College of Health and Professional Studies in Belgrade, Palmira Toljatija 32/6, 11 070 Novi Beograd, Serbia. E-mail: sgoras@yahoo.com

Introduction

Testosterone was synthesized in laboratory in 1931 for the first time, thus allowing clinical experiments with this hormone [1]. Its use has been around for more than 80 years, and there is much more experience behind the use of testosterone than for some newer medicines physicians prescribe today. The use of stimulative substances with the aim of building up muscle mass and improving sports results spread like wildfire among sports competitors during the sixties and seventies of the last century. During that time, stimulative substances were unknown outside the locker rooms and little was done to prevent their use. Today, the anti-steroid movement is very strong. Hardly a day passes without some information being published about the dangers connected to their use. At the same time, the use of steroids aimed at improving physical performances has never been higher. The fact that doping is no longer limited to elite athletes is particularly dangerous. Numerous studies report on doping use being found among the young in amateur and school sports. Many of them use anabolic steroids rather to improve their body looks than to have more success in competitions [2–8].

In the last couple of years, there was a significant shift in researches on doping – from discovery and secondary prevention to primary prevention through education [9]. Important components of these basic prevention strategies are: to identify target groups, to evaluate their knowledge and attitudes concerning doping as well as to determine efficient initial basis for intervention [10]. These studies focus on individuals who do sports and who might benefit from using these substances, on coaches whose task is to provide safety in sports, which is in direct connection to the success of their athletes and on physicians and pharmacists whose medical advice might influence the knowledge of and attitudes towards the use of stimulative substances [11].

Numerous researches were performed among the adolescents in order to obtain data on the stimulative substance misuse, their knowledge of the adverse effects, and their attitudes towards taking prohibited substances. The largest number of researches was performed among the American adolescents, namely the ones doing sports. In the USA, 375,000 of male respondents and 175,000 of female respondents [12] used anabolic-androgenic steroids (AAS) at least once. Other authors who performed their researches in the USA state that the percentage of the AAS users is 6.6% [13]. In Europe, a research performed in six European countries showed that the percentage of high school children using the AAS is 2.1% [14].

The aim of this research was to test the knowledge, attitudes and behaviours of the students at the College of Health and Professional Studies in Belgrade, Serbia regarding the use of stimulative substances and dietary supplements in sports.

Methods

A cross-sectional study was performed at the College of Health and Professional Studies during the summer semester of 2015/16. The size of the respondent group was determined based on the initial parameters: power of the study – 80%, probability of type I error (α) – 0.05, minimum difference in the values of the observed variables – 20%. The research included 321 students at various years of their studies, randomly selected; 34% of which were males. All of the respondents attend College of Health and Professional Studies, which makes 16.2% of the total number of students. The average age of respondents was 21.2 ± 2.1 years (minimum 19, maximum 38, median 24.0 years). The average subject age per gender was not statistically significantly different ($t = 1.344$; $p > 0.05$).

Three departments were included in the survey with 87 (27.1%) medical radiologists, 143 (44.5%) physiotherapists and 91 (28.3%) laboratory technicians. As for their year of studies, there were 89 (27.7%) first-year students, 112 (34.9%) second-year students, and 120 (37.4%) third-year students. It was determined that 87 (27.1%) respondents were born in Belgrade and 234 (72.9%) were from the provinces.

Majority of students were involved in some type of sports activities: volleyball 18.7%, basketball 15.6%, football 14.3% and body building 10%. Prohibited substances were used by 3.7% of male respondents and 2.2% of female respondents. The respondents had 20 minutes before their classes to voluntarily and anonymously fill in the questionaires. All students had the same questions and answered them in the same manner. They were guaranteed discretion for their voluntary and anonymous participation. In order to test the comprehensibility of the questions given, the questionnaire had been validated on a small sample of 12 respondents before being made a part of the research. The questions the students had found incomprehensible were paraphrased and the final version of the questionnaire was determined. The questionnaire contained multiple-choice questions and the respondents answered by circling answers. The exception was the question about the types of stimulative substances and dietary supplements used by them. This question was answered by naming the substance or supplement being used.

Instruments

Surveying the respondents was done by the anonymous epidemiological questionnaire. The respondents gave answers to short questions (both open-ended and closed-ended questions) by writing down relevant information or by choosing from the provided answers. Main research questions were sorted into following four categories: a) sociodemographic data (gender, age, place of birth, family income, sport the respondent is engaged in, current year of studies, exam pass rate and satisfaction with oneself being a student); b) general perception, knowledge of doping substances and methods and knowledge of adverse effects of doping to one's health [9–11]; c) general doping attitudes measured by the Performance Enhancment Attitude Scale (PEAS) [15]. Doping attitude is defined as a predisposition of an individual to use prohibited doping substances and methods. The scale consists of 17 attitude statements which

are measured on a six-point Likert-type scale, ranging from strongly disagree (1/to strongly agree/6). No neutral middle point is offered, and all 17 items are scored in the same direction; d) use of substances and/or supplements in order to enhance sports performance or improve physical appearance.

Statistical data processing

The incomplete questionnaires (9 in total) were not processed. The collected data was reviewed and coded, then processed and presented in tables and charts along with a commentary of the aforementioned, depending on the nature of the observed variable. Description of numerical characteristics in our paper was performed by using classical methods of descriptive statistics, namely by arithmetic mean and median of mean values and as for measures of variability by standard deviation, coefficient of variation and standard error as well as by minimum and maximum values. Relative numbers are used in all tables.

Distribution of numeric variables in our paper was checked by using the Kolmogorov-Smirnov test (normal distribution was tested). The variables that met this criterion, that is, that had normal distribution, were further analysed by parametric methods; non-parametric methods were used on those that did not meet the said criterion.

The analysis of results, depending on the nature of variables themselves, was performed by the Pearson's chi-squared test, in the form of goodness-of-fit test and contingency tables, in order to compare the differences between frequencies of non-parametric characteristics, namely for one or two characteristics.

We used Student's *t*-test for two sets of data to compare the means of the parametric characteristics. As a non-parametric addition to the independent samples, we applied the Rank Sum Test, and to the dependent samples, we used the Wilcoxon signed-rank test.

When performing the linkage analysis of the characteristics, we used methods of one-tailed parametric correlation and regression as well as the non-parametric correlation, depending on the data distribution.

For analysis purposes, three scores were defined: score 1 represents the points won on that part of the questionnaire concerning general knowledge of doping where higher number of points meant greater knowledge, score 2 for the knowledge of side effects where higher number of points meant greater knowledge of effects, and score 3 concerning the attitude towards doping (substances and supplements for strength enhancement) where higher number of points meant more pronounced positive attitude towards doping.

In all analytical methods applied the significance level was set at 0.05.

Program SPSS 20.0 of the Department for Medicinal Statistics and Informatics, Faculty of Medicine in Belgrade, was used to make a data base and process the data.

Results

Students' knowledge of and attitudes towards the use of prohibited substances and supplements in sports

"Good knowledge" was demonstrated by 31.2% of the respondents for general knowledge of doping and by 34.2% of the respondents for knowledge of doping and doping side effects.

The descriptive statistical values for calculated scores are shown in Table 1.

The average score comparison according to gender of our respondents showed there was a statistically highly significant difference in all three scores; the scores for general knowledge of doping and knowledge of doping side effects were higher in the female respondents, whereas the male respondents had higher average scores for attitudes towards doping (Table 2).

Table 1

Descriptive statistical values for calculated scores for 321 respondents

Variable	Minimum–Maximum	Med	Mean ± SD
General knowledge of doping	0.00–16.00	12.00	11.34 ± 2.63
Knowledge of doping side effects	0.00–23.00	14.00	13.82 ± 4.77
Attitudes towards doping	17.00–74.00	34.00	35.48 ± 13.99

Med – median; SD – standard deviaton.

Table 2

Score comparison per gender (109 males and 212 fameles)

Gender	Mean ± SD	SE	*t*	*p*
General knowledge of doping				
male	10.75 ± 2.46	0.23		
female	11.64 ± 2.67	0.18	2.914	0.004**
Knowledge of doping side effects				
male	12.46 ± 4.56	0.43		
female	14.51 ± 4.74	0.32	3.715	0.000**
Attitudes towards doping				
male	39.12 ± 14.29	1.36		
female	33.60 ± 13.48	0.92	3.402	0.001**

SD – standard deviation; SE – standard eror; *p* – statistical significance.

Average score comparison per department of our respondents showed there was a statistically significant difference in the scores achieved for general knowledge of doping and attitudes towards doping: laboratory technicians had the highest average scores, that is, the greatest knowledge of doping, and physioterapists had the poorest general knowledge. The medical radiologists had the lowest score for attitudes towards doping which means that they had the most pronounced negative attitude towards doping, and the highest score was achieved by the physioterapists who, therefore, had the least pronounced negative attitude towards doping. There was no statistically significant difference in knowledge of doping side effects scores per department (Table 3).

The analysis of the average score values per subjects' study year showed that there was a statistically significant difference only in the attitudes on doping score, wherein the subjects in the second and the third year of studies had lower, and the first-year students had the highest average values. No statistically significant differences between study years were recorded in the score values of the general knowledge and knowledge of doping side effects (Table 4).

The analysis of the average score values regarding the habit of engaging in sports showed that there was no statistically significant difference in the average values among three observed scores.

The results of the score comparison regarding a way of engagement in sports activities are shown in Table 5.

Table 3

Score comparison per department

Study department	n	Mean ± SD	95% CI		Minimum–Maximum	f	*p*
			Bottom limit	Upper limit			
General knowledge of doping							
medical radiologist	87	11.59 ± 2.52	11.06	12.13	0.00–14.00		
physiotherapist	143	10.75 ± 2.80	10.39	11.42	0.00–15.00		
laboratory technician	91	11.70 ± 2.39	11.20	12.20	6.00–16.00	2.819	0.049*
Total	321	11.34 ± 2.63	11.05	11.63	0.00–16.00		
Knowledge of doping side effects							
medical radiologist	87	14.50 ± 3.92	13.66	15.34	0.00–23.00		
physiotherapist	143	13.80 ± 5.06	12.96	14.64	0.00–20.00		
laboratory technician	91	13.49 ± 5.00	12.15	14.23	0.00–21.00	1.676	0.189
Total	321	13.82 ± 4.77	13.29	14.34	0.00–23.00		
Attitudes towards doping							
medical radiologist	87	31.21 ± 12.62	28.52	33.90	17.00–67.00		
physiotherapist	143	38.57 ± 14.45	36.18	40.96	17.00–74.00		
laboratory technician	91	34.70 ± 13.45	31.90	37.50	17.00–68.00	8.005	0.000**
Total	321	35.48 ± 13.99	33.94	37.01	17.00–74.00		

SD – standard deviation; CI – confidence interval; *p* – statistical significance.

Table 4

Score comparison per study year (from the first to third year)

Study year	n	Mean ± SD	95% IP for the average		Minimum–Maximum	f	*p*
			Bottom limit	Upper limit			
General knowledge of doping							
first year	89	11.05 ± 2.66	10.49	11.61	4.00–15.00		
second year	112	11.16 ± 2.92	10.61	11.70	0.00–15.00		
third year	120	11.72 ± 2.27	11.31	12.13	3.00–16.00	2.073	0.127
Total	321	11.34 ± 2.63	11.05	11.63	0.00–16.00		
Knowledge of doping side effects							
first year	89	13.66 ± 5.01	12.60	14.71	0.00–20.00		
second year	112	14.21 ± 4.68	13.33	15.09	0.00–23.00		
third year	120	13.57 ± 4.69	12.72	14.42	0.00–21.00	0.586	0.557
Total	321	13.82 ± 4.77	13.29	14.34	0.00–23.00		
Attitudes on doping							
first year	89	38.91 ± 15.57	35.63	42.19	17.00–73.00		
second year	112	33.90 ± 13.78	31.32	36.48	17.00–67.00		
third year	120	34.41 ± 12.55	32.14	36.68	17.00–74.00	3.798	0.023*
Total	321	35.48 ± 13.99	33.94	37.01	17.00–74.00		

SD – standard deviation; IP – interpercentile range; *p* – statistical significance.

Table 5

Score comparison according to the role of engaging in sport activities

Role in sport activites	Mean ± SD	SD	SE	t	*p*
General knowledge of doping					
first team member	19 ± 11.36	1.89	0.43		
recreationally active	248 ± 11.19	2.66	0.16	0.274	0.784
Knowledge of doping side effects					
first team member	19 ± 13.10	4.40	1.01	0.423	0.673
recreationally active	248 ± 13.59	4.87	0.30		
Attitudes on doping					
first team member	19 ± 34.57	12.31	2.82		
recreationally active	248 ± 36.01	13.85	0.87	0.438	0.662

SD – standard deviation; SE – standard error; *p* – statistical significance.

Table 6

Score comparisons with regards to the question:
"Do you think that use of substances to improve efficiency in sports is unethical?"

Scores answers to the question	n	Mean ± SD	SE	t	*p*
General knowledge of doping					
yes	250	11.42 ± 2.57	0.16		
no	69	11.01 ± 2.87	0.34	1.153	0.250
Knowledge of doping side effects					
yes	250	14.19 ± 4.70	0.29		
no	69	12.59 ± 4.88	0.58	2.478	0.014*
Attitudes on doping					
yes	250	30.18 ± 11.31	0.84		
no	69	34.04 ± 15.62	1.88	-3.115	0.002**

SD – standard deviation; SE – standard error; *p* – statistical significance.

General perception of the use of substances and/or supplements for the increase of strength and muscle definition in sports

The results shown in Table 6 represent the score comparisons with regards to the question: "Do you think that use of substances to improve efficiency in sports is unethical?"

The analysis of the average score values regarding the attitude on the ethics of the use of substances to improve efficiency in sports in our subjects, showed that there was a statistically significant difference in the average score values for the knowledge of the side effects of and attitudes towards doping; therefore, the subjects who considered that the use of substances for improvement purposes was unethical, also knew more about them, but at the same time they had more distinct negative attitude on doping. The score values for general knowledge of doping did not significantly differ from the answers to the question: "Do you think that use of substances to improve efficiency in sports is unethical?"

Furthermore, answering the question about the sources they like using the most to obtain information on doping, our subjects stated that most commonly they obtain information on doping from books (71%), their pharmacists (71.3%), which was followed by personal trainers (69%), personal physician (67.2%), and the internet (65.3%). The interesting thing is that learning about doping from parents was not even within the top five stated sources, with only 55.4% of the answers. When the average values of three analysed scores of the first three sources were compared, no statistically significant difference were observed.

Discussion

The results of this research provided certain information on attitudes and knowledge of students from three study departments (physiotherapeutists, laboratory technicians and medical radiologists) regarding the problem of the use of stimulative substances in sports. The research may be of importance due to the population covered by the research, since it is the young population whose priority was success or better appearance, while their health was of secondary importance.

In scoring the answers to questions on general knowledge of doping and knowledge of side effects, the answers were rated cumulatively.

Blank et al. [16] stated that in their research they received almost identical results. The comparison of general knowledge as well as knowledge of side effects depending on the gender of our subjects, showed that female subjects presented greater general knowledge on prohibited substances and greater knowledge on adverse effects to the organism than the male subjects. Students' attitudes regarding the use of stimulative substance and dietary supplements showed higher average values in male respondents, which indicated that they have less distinct negative attitude towards doping than female respondents. The female respondents had greater knowledge of the problems related to doping and side effects when compared to the male respondents.

As opposed to our research, Blanket al. [16] concluded in their research that there was a correlation between gender and knowledge, which could be connected to the fact that there was also a correlation between gender and higher parti-

cipation in sports activities. Male subjects were more engaged in sports and showed better results in terms of knowledge. Experience in the use of stimulative substances in the preceding period had not significantly affected knowledge. It would seem logical that someone who used stimulative substances has greater knowledge of all such substances.

The comparison of the average score values among the study departments of our respondents showed that the students from the laboratory technicians department had the highest average values i.e., the highest general knowledge of doping while the physiotherapists had the poorest.

The "good knowledge" limit point was set at 80% of correct answers to the asked questions [15]. In comparison between general knowledge and the use of stimulative substances, one third of the non-using respondents and respondents using stimulative substances showed "good knowledge" and the limit point was a result of greater general knowledge of doping among the non-using respondents. In comparison between general knowledge and the use of stimulative supplements, the non-using and supplement using respondents showed "good knowledge" and worse knowledge, respectively, and the limit point was a result of greater general knowledge of doping side effects among the respondents not using supplements.

Comparing the knowledge of the adverse effects and the use of the strength increase substances, the non-using respondents showed better knowledge than the respondents using prohibited substances, and the limit point was a result of approximately equal number of correct answers to the questions regarding the respondents' general knowledge. Comparing the knowledge of adverse effects and the use of supplements, the non-using respondents showed better knowledge than the respondents using supplements, and the limit point was a result of greater general knowledge of doping side effects among the respondents not using supplements.

Other researchers found bigger differences in knowledge the section of general knowledge of doping and between in the section of knowledge of doping side effects [16].

These values of knowledge scores were a strong invitation for further research on the factors which affect general knowledge of doping and knowledge of side effects, which ought to be included in the pre-emptive measures for educational purposes.

Knowledge and attitudes of student population regarding the problem of doping in sports were research subjects of certain authors. Melia et al. [17] conducted a survey of five Canadian regions including 107 schools in order to determine the prevalence of the use of anabolic-androgenic steroids, their attitudes and knowledge about doping. The results showed that many of them used prohibited substances in the year prior to the survey, and that a significant number of respondents stated that they were using other substances in attempts to improve sports results. The results were alarming and unexpected for teachers, healthcare and sports professionals.

In score values for general knowledge and knowledge on side effects, no statistically significant differences were recorded between the study years; nevertheless, the difference was registered in the score increase between the study years. This surely indicated that a *curriculum* content was not sufficient for a significant change of the required knowledge.

In analysis of the attitudes towards the use of stimulative substances and dietary supplements, we found higher average values in the male respondents. So, the female respondents had greater knowledge of the problems related to prohibited substances as well as side effects, and the male respondents had less distinct negative attitude towards doping than the female respondents.

The results on the attitude scale largely depended on statements, and this may lead to underestimated results since the respondents hesitated to respond honestly. Even in conditions of anonymity, respondents may respond in a manner they believe to be socially desired or expected. Correlations found in this research are significant, but not sufficient, which indicates the fact that there are other unidentified factors which could contribute to a greater knowledge and attitudes regarding the use of stimulative substances.

Our respondents' study years showed that a statistically significant difference exists only when comparing score values for the attitudes, where the students in the second and the third year of studies had lower, and the first-year students had the highest average values. Such a result means that poorly expressed negative attitude on doping at the beginning of the studies is slowly corrected and improved in the subsequent study years, where students have more and more distinct negative attitude on doping, and the physiotherapists had the highest values, as they have the least negative attitude on doping.

The analysis of the average score values for the attitude of our respondents on the ethics of use of substances to improve efficiency in sports, showed that there was a statistically significant difference between the average score values of the knowledge on side effects and attitudes. Students, who considered that the use of substances for improvement was unethical, also knew more about their side effects and had a distinctly more negative attitude on doping. General knowledge of the prohibited substances did not significantly affect perception of ethics.

The students with greater general knowledge on prohibited substances showed better general perception in understanding frequency of use of the substance to improve efficiency in sports. Adoption of the new global Anti-Doping Code in 2015 resulted in altered rules. Today, it is clear that one cannot possibly test all sports in the same manner and that changes ought to be made to the manner as well as to the approach to the fight against doping. People and their perception of doping are much more important than new analytical methods. The basis of the doping problem is primarily harmfulness of the effects to certain organ systems caused by the use of doping substances; young people should be especially warned about this [18].

Most common sources used by our students to obtain information on doping, although their selection did not affect the knowledge and attitudes about doping, were printed media, books, pharmacists and personal trainers as well. Slightly smaller but still significant number of reports that

were personal physician and internet. The interesting thing is that learning about doping from parents was not even within the top five stated sources.

In their research, Blank et al. [16] stated that the majority of respondents sought information on the prohibited substances on the internet in publications and the least often from physicians. As for the supplements, a high number of the respondents considered that they had insufficient information on supplements and the majority of the respondents stated that they obtained information from multiple sources. The students who obtained information from a single source mainly referred to media as the source of data on dietary supplements. The internet was the main source of information about dietary supplements for students in Poland [18]. Since a large number of respondents obtain information from the media and from friends, the greatest attention should be paid to promotion of proper use of dietary supplements, which should be conducted by physicians and pharmacists. According to foreign research, 72% of physicians and 89% of medical nurses recommend the use of dietary supplements [19].

There are numerous research studies on the parents' role in the behaviour and attitude modulation in terms of prevention of a high-risk behaviour related to sexual behaviour and smoking [20]. Nevertheless, there are no studies to deal with the parents' impact on the behaviour of children in connection with some other forms of high-risk behaviour, such as doping. A purpose of such researches would be to assess parents' knowledge and attitudes depending on the child's age, as a first step towards proposing educational and preemptive intervention. Parents were neglected in doping prevention literature; now, with doping interventions shifting towards prevention and education, this type of assessments is needed as well as assessments in order to determine knowledge and current education status of target groups, such as parents. Based on the previous research, emphasis of future educational campaigns should be put on the contents about doping effects on health.

It is important to point out that results obtained in this research are significant, but not sufficient which indicates that there are other unidentified factors which could contribute to greater knowledge and better attitudes regarding the use of stimulative substances.

This study has some limitations, such as: the research was not performed in all departments of the College of Health and Professional Studies; questionnaires were completed under the tutor's supervision, which naturally resulted in higher rate of desirable answers. Beside that the survey was anonymous, the respondents personally handed in the completed questionnaires to the person performing the survey.

Conclusion

The use of stimulative substances is not only a problem of elite sportsmen, but it also exists in the general population and represents a general social problem.

The results obtained from the student population with regard to the attitudes on the use of prohibited substances, knowledge and informedness of doping adverse effects, justify further similar researches.

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Complete percutaneous myocardial revascularization in patients with STEMI complicated by cardiogenic shock

Kompletna perkutana revaskularizacija miokarda kod bolesnika sa STEMI komplikovanim kardiogenim šokom

Milovan Petrović*†, Milana Jaraković†, Milenko Čanković*†, Ilija Srdanović*†, Mila Kovačević*†, Dragica Tešić*, Vladimir Ivanović*†, Aleksandar Redžek*†, Lazar Velicki†

University of Novi Sad, *Faculty of Medicine, Novi Sad, Serbia;
†Institute of Cardiovascular Diseases of Vojvodina, Sremska Kamenica, Serbia

Abstract

Background/Aim. Despite considerable progress in terms of early myocardial revascularization and the use of mechanical circulatory support, cardiogenic shock continues to be the leading cause of death in acute myocardial infarction. The current recommendations of the European Society of Cardiology advocate early revascularization of all critical stenosis or highly unstable lesions in the state of cardiogenic shock, while recently published studies favour the early revascularization of the infarct related artery only, in patients with acute myocardial infarction with the ST segment elevation (STEMI) presenting with cardiogenic shock. The aim of the study was to assess the impact of the complete early percutaneous myocardial revascularization in an acute myocardial infarction complicated by cardiogenic shock on intra-hospital mortality. **Methods**. The research was conducted as a retrospective observational analysis of data obtained from the hospital registry for cardiogenic shock. The study group consisted of 235 patients treated in the period from August 2007 until October 2016 for STEMI complicated by cardiogenic shock. Three groups were formed. The first group consisted of patients with one vessel disease who underwent revascularization of infarct related artery; the second group of patients had multi-vessel disease and only culprit lesions were revascularized and the third one consisted of patients with multi-vessel disease and the complete myocardial revascularization performed. Additional subgroups were formed in reference to the intra-aortic balloon pump (IABP) implantation. Intra-hospital mortality was analyzed in all groups and subgroups. **Results.** Revascularization of the culprit lesion alone among patients with multivessel disease was performed in 142 (60.4%) patients while the complete revascularization (revascularization of "culprit" and other significant lesions) was performed in 28 (11.9%) patients with multi-vessel disease. There were 65 (27.7%) patients with single-vessel disease who underwent revascularization of infarct related artery. The lowest mortality was found in the group of patients with multi-vessel coronary disease who underwent complete myocardial revascularization and had IABP implanted (mortality was 35.7%). The difference in the mean value of the left ventricular ejection fraction (EF) between the surviving and deceased patients was statistically significant ($p < 0.005$). The average EF of survivors was 44% (35%–50%) while 30% (25%–39.5%) deceased of patients. Based on the obtained data, the mathematically predictive model was tested. The receiver operating characteristic (ROC) curve showed that our model is a good predictor of fatal outcome ($p < 0.0005$; AUROC = 0.766) with the sensitivity of 80.3%, and the specificity of 67%. **Conclusion**. STEMI complicated by cardiogenic shock is still associated with a high mortality rate. Complete myocardial revascularization independently as well as in combination with an IABP, significantly reduces mortality in patients with acute STEMI complicated by cardiogenic shock.

Key words:
myocardial infarction; shock, cardiogenic; myocardial revascularization; percutaneous coronary intervention; mortality.

Apstrakt

Uvod/Cilj. I pored značajnog napretka rane miokardne revaskularizacije i upotrebe mehaničke cirkulatorne podrške, kardiogeni šok i dalje predstavlja vodeći uzrok smrti kod akutnog infarkta miokarda. Aktuelne preporuke Evropskog udruženja kardiologa preporučuju ranu revaskularizaciju svih kritičnih stenoza ili visoko nestabilnih lezija kod stanja kardiogenog šoka, dok skorašnje studije preporučuju ranu revaskularizaciju samo infarktne koronarne arterije kod bo-

Correspondence to: Milovan Petrović, Institute of Cardiovascular Diseases Vojvodina, Put doktora Goldmana 4, 21 204 Sremska Kamenica, Serbia. E-mail: milovan.petrovic@mf.uns.ac.rs

lesnika sa akutnim infarktom miokarda sa ST segment elevacijom (STEMI) i kardiogenim šokom. Cilj studije bio je da analizira uticaj kompletne rane perkutane miokardijalne revaskularizacije u akutnom infarktu komplikovanim kardiogenim šokom na intrahospitalni mortalitet. **Metode.** Istraživanje je sprovedeno kao retrospektivna opservaciona analiza podataka dobijenih iz bolničkih registara za kardiogeni šok. Studijska grupa imala je 235 bolesnika lečenih u periodu od avgusta 2007. do oktobra 2016. godine zbog STEMI komplikovanim kardiogenim šokom. Formirane su tri grupe. Prva grupa sastojala se od bolesnika sa bolešću jednog krvnog suda podvrgnutih revaskularizaciji, arterije povezane sa infarktom. Drugu grupu činili su bolesnici sa multisudovnom bolešću, kod kojih je samo revasulizirana *culprit* lezija, a treću grupu činili su bolesnici sa multi-sudovnom bolešću i kompletnom revaskularizacijom miokarda. Dodatne subgrupe formirane su na osnovu implantaciju intraaortne balon pumpe (IABP). Intrahospitalni mortalitet analiziran je u svim grupama i subgrupama. **Rezultati.** Revaskulsrizacija samo *culprit* lezije kod bolesnika sa multisudovnom bolešću učinjena je kod 142 (60,4%) bolesnika, dok je kompletna revaskularizacija (revaskularizacija *culprit* i ostalih značajnih lezija) kod bolesnika sa multisudovnim lezijama učinjena kod 28 (11,9%) bolesnika. Šezdeset pet (27,7%) bolesnika sa jednosudovnom bolešću podvrgnuto je revaskularizaciji infarktne arterije. Najniži mortalitet bio je u grupi bolesnika sa multisudovnom koronarnom bolešću koji su podvrgnuti kompletnoj revaskularizaciji i implantaciji IABP (mortalitet 35,7%). Razlika u srednjoj vrednosti ejekcione frakcije leve komore (EF) između preživelih i umrlih bila je statistički značajna ($p < 0,005$). Prosečna vrednost EF kod preživelih bila je 44% (35%–50%), dok je kod umrlih iznosila 30% (25%–39,5%). Na osnovu korišćenih podataka, testiran je matematički prediktivni model. *Reciever operating characteristics* (ROC) kriva pokazala je da je naš model dobar pokazatelj fatalnog ishoda ($p < 0,0005$; AUROC = 0,766), uz senzitivnost od 80,3% i specifičnost 67%. **Zaključak.** STEMI komplikovan kardiogenim šokom još uvek je udružen sa visokom stopom mortaliteta. Samo kompletna revaskularizacija miokarda, kao i u kombinaciji sa IABP, značajno smanjuje mortalitet kod bolesnika sa akutnim STEMI komplikovanim kardiogenim šokom.

Ključne reči:
infakrt miokarda; šok, kardiogeni; miokard, revaskularizacija; perkutana koronarna intervencija; mortalitet.

Introduction

Cardiogenic shock is the state of critical tissue hypoperfusion, resulting from cardiac failure which is in 75% of cases caused by acute myocardial infarction [1–3]. The incidence of cardiogenic shock in acute myocardial infarction is 5%–15% [4].

Despite the significant therapeutic progress, primarily in the sense of early myocardial revascularization strategy and aggressive inotropic and vasopressor support, cardiogenic shock continues to be the leading cause of death in acute myocardial infarction with the mortality rate of 42%–48% [5–9].

Data obtained from a limited number of studies show a possible reduction in hospital mortality related to cardiogenic shock, which is associated with the strategy of performing early myocardial revascularization in this group of patients [4].

The current European Guidelines on Myocardial Revascularization recommend early myocardial revascularization either by percutaneous coronary intervention or coronary artery bypass grafting (CABG). Also, revascularization of culprit lesion and all critical stenosis or highly unstable lesions was highly encouraged in cardiogenic shock at that time by the guideline [2].

When it comes to the use of an intra-aortic balloon pump (IABP) in cardiogenic shock caused by acute myocardial infarction, the valid recommendation of the European Society of Cardiology (ESC) and the American College of Cardiology/American Heart Association (ACC/AHA) guidelines do not recommend routine IABP implantation, except in the case of mechanical complications of acute myocardial infarction, in order to bridge to surgery [10, 11].

Methods

The research was conducted as a retrospective observational analysis of data obtained from the hospital information system of the Institute of Cardiovascular Diseases of Vojvodina in Sremska Kamenica. The study group consisted of 235 patients with ST-elevation myocardial infarction (STEMI) complicated by cardiogenic shock who were treated in the period from August 2007 until October 2016. The inclusion criterion was the STEMI of any localization with signs of cardiogenic shock according to the current guidelines of the ESC as well as urgent coronarography and primary percutaneous coronary intervention (pPCI) [10].

Patients in whom cardiogenic shock developed before urgent coronarography were analyzed. The criteria used for the diagnosis of cardiogenic shock (Killip Class IV) were: systolic blood pressure less than 90 mmHg lasting longer than 30 minutes, or the necessity of vasopressor therapy in order to achieve systolic arterial blood pressure value ≥ 90 mmHg; pulmonary congestion or elevated left ventricular filling pressures; signs of tissue perfusion disorder with at least one of the following criteria: altered mental status, cold, sticky skin, oliguria (< 0.5 mL/kg/h); elevated serum lactate (> 1.5 mmol/L) [10–13]. Patients who failed pPCI or had fatal outcome during intervention were not included in the analysis.

Total ischemic time could not be determined since these patients were already in a severe general condition and cardiogenic shock at admission, and the data on the onset of symptoms were unreliable or impossible to obtain.

The primary goal was to assess the impact of the complete early percutaneous myocardial revascularization in acute myocardial infarction complicated by cardiogenic shock on intra-hospital mortality, while the secondary goal

was to assess the impact of the intra-aortic balloon pump (IABP) in cardiogenic shock caused by acute myocardial infarction on intra-hospital mortality.

As part of the patient admission and preparation process for PCI, all patients were admitted to the Coronary Care Unit, where clinical examination and electrocardiography (ECG) were performed and blood was taken to determine the markers of myocardial necrosis. All patients were sedated, endotracheally intubated and placed on invasive mechanical ventilation. All patients were given the circulatory support in terms of vasopressors and/or inotropes. After an adequate preparation, urgent coronarography and pPCI were performed in all patients at the catheterization laboratory (Cath Lab). Depending on a patient's clinical condition, echocardiography was performed before or after percutaneous coronary intervention.

Complete percutaneous coronary myocardial revascularization involved not only mechanical revascularization of culprit lesions but also other critical stenoses in both the infarct and other coronary arteries. The method of revascularisation of only „culprit lesions", or complete revascularization was decided by the interventional cardiologist during the procedure depending on the form and type of a lesion, regarding its suitability and feasibility for the complete revascularization.

Implantation of the intra-aortic balloon pump (IABP Datascope, Corp Mahwah, NJ USA) was assessed and decided on by the interventional cardiologist according to clinical condition of the patient. Since this was a retrospective follow-up, there were no clearly defined criteria for the IABP implantation, so the decision on implantation was made by the interventional cardiologist.

All implantations were performed at the Cath Lab during or after the percutaneous coronary intervention PCI procedure.

Accordingly, three large groups were formed. The first group consisted of patients with one vessel disease who underwent revascularization of infarct related artery; the second group was made up of patients with multivessel disease and only culprit lesions revascularized, and the third one consisted of patients with multi-vessel disease and the complete myocardial revascularization performed. Additional subgroups were formed within these three large groups in reference to the IABP implantation. Intra-hospital mortality was analyzed in all groups and subgroups. The patients with unsuccessful revascularization of culprit lesion were excluded from the analysis.

In this paper, the following measures of the descriptive statistics were used: arithmetic mean, standard deviation, median, quartiles, frequencies, and percentages. For the comparison of the mean values of variables of two populations, the independent samples *t*-test and the Mann-Whitney test were used. The correlation of the categorical variables was examined using the Chi-square (χ^2) test for the contingency tables or the Fisher test. The effects of variables on the treatment outcome were determined using the univariate and multivariate binary logistic analyses. Determining the outcome prediction markers was assisted by the recieves operating characteristic (ROC) curves. The Kaplan Meier and Cox regression analyses were used for the statistical analysis of survival.

Statistical analysis and data processing were done using the Statistical Package for Social Sciences – SPSS program for Windows, Version 17.0 (SPSS Inc. Chicago, IL), in which the significance limit was $p < 0.05$.

Results

This study included 235 patients, 137 (58.3%) men and 98 (41.7%) women. The average age of men was 64.94 ± 10.87 years while the average age of women was 68.87 ± 11.05 years. Demographic characteristics, risk factors for ischemic heart disease, and early history of myocardial infarction of the patients included in the study are shown in Table 1. Urgent coronarography and then primary PCI were performed in all patients. Urgent coronarography found 65 (27.7%) patients with single-vessel coronary disease and 170 (72.3%) patients with multi-vessel coronary disease. One hundred and twenty-seven (54%) patients had anterior wall STEMI, while 108 (46%) patients had inferior wall STEMI. Revascularization of the culprit lesion alone among patients with multi-vessel disease was performed in 142 (60.4%) patients, while the complete revascularization (revascularization of „culprit" and other significant lesions) among patients with multi-vessel disease was performed in 28 (11.9%) patients. There were 65 (27.7%) patients with single-vessel disease who underwent revascularization of infarct related artery.

Intra-aortic balloon pump was implanted in 84 (35.7%) patients (Table 1), predominantly in patients with anterior wall STEMI (75%) and in 25% of patients with the inferior wall STEMI. IABP was significantly more often implanted in patients with the anterior wall STEMI ($p = 0.0005$). Mortality in relation to the type of coronary disease and the implantation of IABP is shown in Figure 1. There was a statistically significant difference in the mortality rate among the groups ($p = 0.046$), and the lowest mortality was found in the group of patients with multi-vessel coronary disease who underwent complete myocardial revascularization and IABP was implanted (mortality was 35.7%). However, the Kaplan-Meier survival curve and Cox regression analysis showed that there are no statistically significant difference ($p = 0.220$) in survival length between the groups (Figure 2).

The intra-hospital mortality rate of the sample was 62.6% (Table 1). Implantation of IABP ($p = 0.406$), type of infarction ($p = 0.171$) as well as whether it was single or multi-vessel coronary disease ($p = 0.341$) did not significantly affect mortality. Mechanical complications of STEMI appeared as ventricular septal defect in 5 (2.1%) patients, rupture of the papillary muscle of the mitral valve also in 5 (2.1%) patients and as the tamponade due to the rupture of the free wall of the myocardium in 1 (0.4%) patient. The total mortality of patients with mechanical complication was high (90.9%), but there was no statistically significant difference in regards to the mortality of patients without mechanical complication ($p = 0.219$).

Table 1

Demographic and clinical characteristics of patients

Variable	All patients (n = 235)	Single-vessel (n = 65)	Multi-vessel and complete revas-cularization (n = 28)	Multi-vessel and incomplete re-vascularization (n = 142)	*p*
Sex, n (%)					
male	137 (58.3)	38 (27.7)	25 (18.2)	74 (54.1)	0.001
female	98 (41.7)	27 (27.5)	3 (3.1)	68 (69.4)	
HTA, n (%)	137 (58.3)	37 (27)	14 (10.2)	86 (62.8)	0.425
DM, n (%)	64 (27.2)	13 (20.3)	8 (12.5)	43 (67.2)	0.333
HLP, n (%)	44 (18.7)	7 (15.9)	9 (20.5)	28 (63.6)	0.066
Smoking, n (%)	75 (31.9)	20 (26.7)	6 (8)	49 (65.3)	0.326
Heredity, n (%)	44 (18.7)	16 (36.4)	6 (13.6)	22 (50)	0.249
Previous MI, n (%)	17 (7.2)	3 (17.6)	1 (5.9)	13 (76.5)	0.202
Age (years), mean (range)	69.0 (59.0–75.0)	68.0 (58.5–73.0)	64.5 (55.5–73.0)	70.0 (61.0–76.0)	0.237
EF (%), mean (range)	36.0 (27.0–46.0)	39.0 (30.0–48.0)	35.0 (21.5–44.5)	35.0 (27.0–46.0)	0.249
Localization, n (%)					
inferior	108 (46.0)	26 (24.1)	11 (10.2)	71 (65.7)	0.307
anterior	127 (54.0)	39 (30.7)	17 (13.4)	71 (55.9)	
Mechanical complications, n (%)					
None	224 (95.2)	61 (93.9)	28 (100)	135 (95.1)	
VSD	5 (2.1)	3 (4.6)	0 (0)	2 (1.4)	0.598
RPM	5 (2.1)	1 (1.5)	0 (0)	4 (2.8)	
Tamponade, n (%)	1 (0.4)	0 (0)	0 (0)	1 (0.7)	
TIMI Flow, n (%)					
0	20 (8.5)	4 (20)	3 (15)	13 (65)	
1	9 (3.8)	6 (66.7)	0 (0)	3 (33.3)	0.215
2	25 (10.6)	7 (28)	0 (0)	16 (64)	
3	181 (77)	48 (26.5)	23 (12.7)	110 (60.8)	
TIMI Code, n (%)					
TF < 3	54 (23)	17 (31.5)	5 (9.3)	32 (59.3)	0.670
TF = 3	181 (77)	48 (26.5)	23 (12.7)	110 (60.8)	

EX – died; IABP – intra-aortic balloon pump; HTA – arterial hypertension; DM – diabetes mellitus; HLP – hyperlipidaemia; MI – myocardial infarction; EF – ejection fraction; VSD – ventricular septal defect; RPM – rupture of papillary muscle; TIMI – thrombolysis in myocardial infarction.

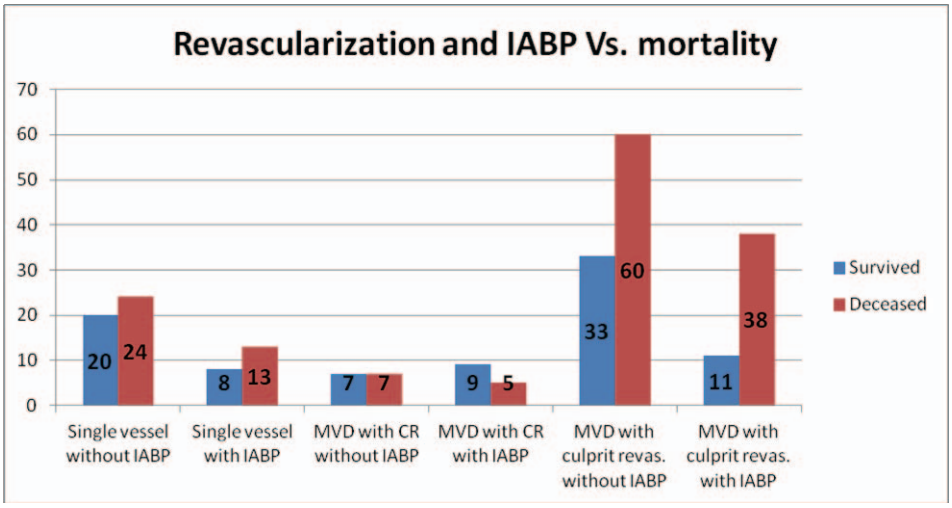

Fig. 1 – Percutaneous myocardial revascularization, intra-aortic balloon pump (IABP) use and mortality.
MVD – multivessel disease; CR – complete revascularization.

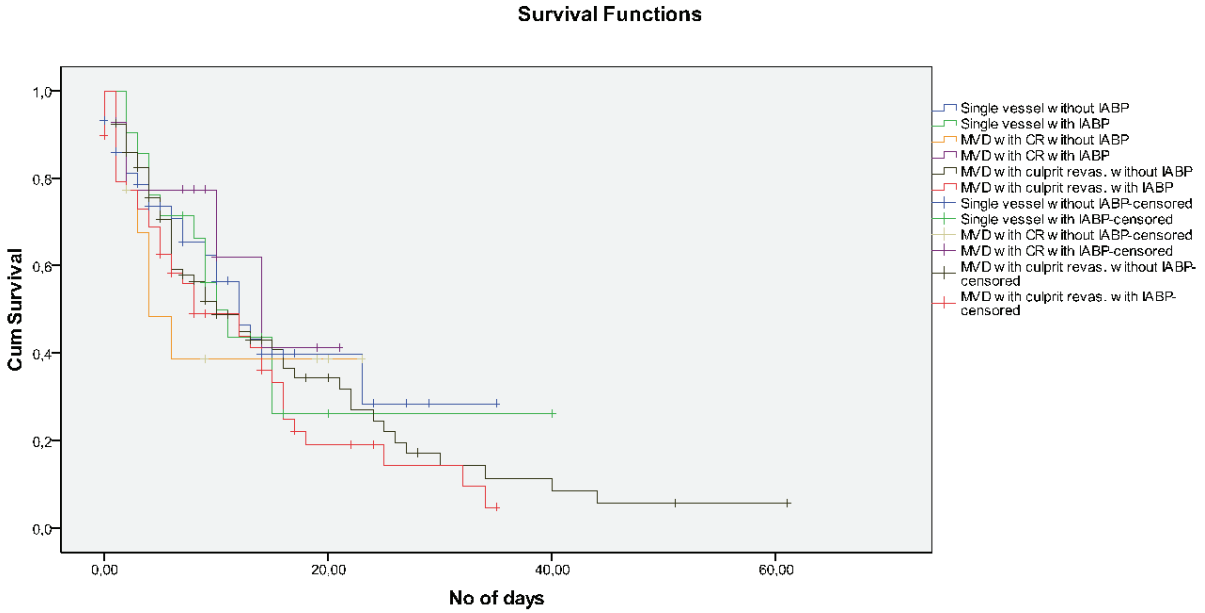

Fig. 2 – Kaplan Meier Curve of intra-hospital survival in relation to the type of coronary disease and revascularization; MVD – multi-vessel disease; CR – coronary revascularization; ($p > 0.05$).

Table 2

Age, EF, complete percutaneous revascularization, TIMI flow and influence on mortality

Variable	Univariate binary logistic regression		Multivariate binary logistic regression	
	OR (95%CI)	*p*-value	OR (95%CI)	*p*
Age (year)	1.029 (1.005–1.054)	0.019	1.035 (1.007–1.064)	0.015
EF (%)	0.928 (0.904–0.953)	< 0.0005	0.924 (0.899–0.950)	< 0.0005
Complete revascularization	0.500 (0.291–0.858)	0.012	0.413 (0.222–0.767)	0.005
TIMI Flow	0.395 (0.195–0.800)	0.010	0.409 (0.183–0.916)	0.030

EF – left ventricular ejection fraction, TIMI – thrombolysis in myocardial infarction.
OR – odds ratio; CI – confidence interval.

Table 3

ROC curve – Age and ejection fraction in the fatal outcome prediction

Parameters	ROC curve				
	Area (95% CI)	Cut-off	Sensitivity	Specificity	*p*
Age (year)	0.598 (0.523–0.672)	65.5	63.3%	51.1%	0.012
Ejection fraction (%)	0.728 (0.660–0.796)	38.5	72.1%	68.2%	< 0.005

ROC – reciever operating characteristic; CI – confidence interval.

In the investigated group of patients, age affected the occurrence of fatal outcome ($p = 0.015$) (Table 2). The average age of the survivors was 64.5 (56–73) years and the deceased 70 (62–76) years. The odds ratio for age was 1.035 (1.007–0.064), which means that the increase of 1 year in life age increases the risk of fatal outcome by 3.5%. The ROC curve showed that age of patients was not a good marker for the prediction of fatal outcome (area = 0.598; sensitivity = 63.3%; specificity = 51.1%), (Table 3). The difference in the mean value of the left ventricular ejection fraction (EF) between the surviving and deceased patients was statistically significant ($p < 0.005$), (Table 3). The average EF of survivors was 44% (35%–50%), while it was 30% in the deceased ones (25%–39.5%). The OR for the EF was 0.924 (0.899–0.950). The increase of EF by 1 decreased the risk of death by 7.5%. The ROC curve in Table 3 shows that the EF can be a good marker for the prediction of fatal outcome (area = 0.728; sensitivity = 72.1%: specificity = 68.2%).

The incidence of complete revascularization was significantly lower in the female patients (30.6% in relation to 46% of men, $p < 0.025$) while mortality was significantly higher ($p < 0.0005$).

The quality of TIMI flow after the stent implantation on the "culprit lesion" affected the occurrence of fatal outcome ($p = 0.030$). The OR for TIMI flow was 0.409 (0.183–0.916). The patients with TIMI 3 flow had nearly 60% lower risk of fatal outcome (Table 2).

Based on the obtained data, the mathematically predictive model was tested. The Hosmer-Lemeshow test was performed proving that the model was good ($p = 0.124$). The

ROC curve showed that our model was a good predictor of fatal outcome ($p < 0.0005$; AUROC = 0.766). The cut-off value was 55.8235 while the sensitivity was 80.3%, and the specificity 67% (Figure 3).

The mortality of patients in reference to the type of coronary disease is shown in Table 4. There was a statistically significant difference in mortality rate between the groups ($p < 0.018$). The lowest mortality rate was in the group of patients who had multi-vessel coronary disease and underwent complete myocardial revascularization (42.9%). It is important to note that the sample in this group of patients was smaller than in other two groups. The Kaplan-Meier and Cox regression analyses did not show a statistically significant difference ($p = 0.226$) in the length of survival among the patient groups (Figure 4).

The mortality analysis in relation to TIMI flow after the stent implantation showed that the highest mortality rate was in the patients with TIMI 0 flow (80% of the deceased), while the lowest mortality rate was in the patients with TIMI 3 flow (58% of the deceased). However, there was no statistically significant difference in mortality between the groups ($p = 0.071$) (Table 5).

Table 4
Mortality of patients in reference to the type of coronary disease

Type of coronary disease	Mortality [n (%) within groups]			
	No	Yes	Total	*p*
Singlevessel	28 (43.1)	37 (56.9)	65 (27.7)	
Multivessel and complete revascularization	16 (57.1)	12 (42.9)	28 (11.9)	0.018
Multivessel and incomplete revascularization	44 (31.0)	98 (69.0)	142 (60.4)	

Table 5
Mortality in relation to TIMI flow

TIMI flow	Mortality, n (%)		*p* value
	No	Yes	
0	4 (20)	16 (80)	0.071
1	2 (22.2)	7 (77.8)	
2	6 (24)	19 (76)	
3	76 (42)	105 (58)	

TIMI – thrombolysis in myocardial infarction.

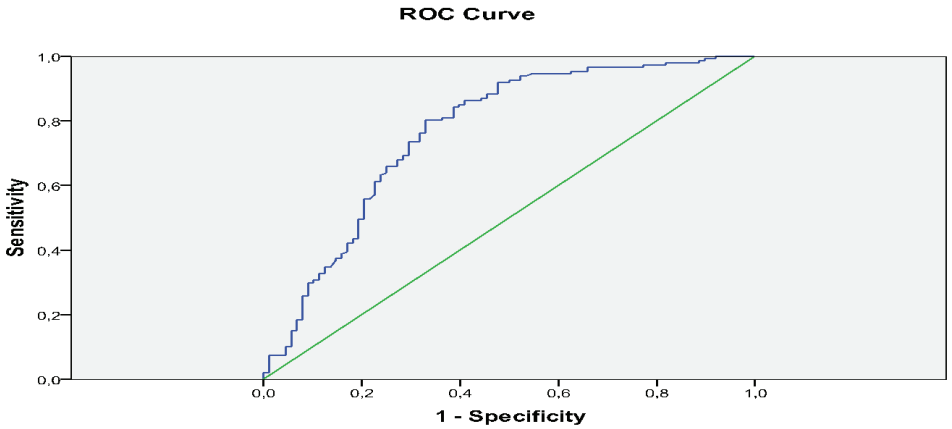

Fig. 3 – Diagonal segments are produced by ties.
ROC – reciever operating characteristic

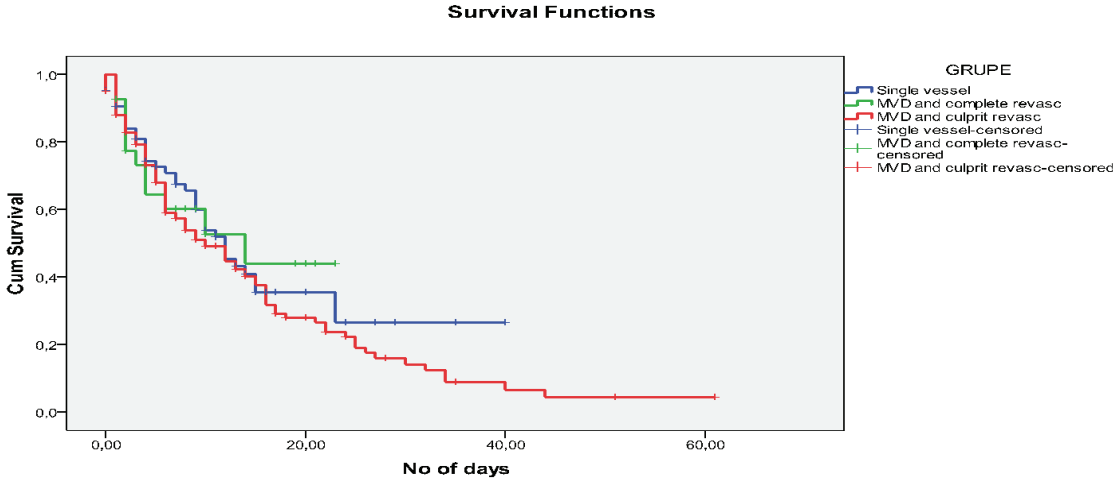

Fig. 4 – Length of survival among the patient groups.

Discussion

Our retrospective research analyzed the influence of the complete early myocardial revascularization in acute myocardial infarction with ST elevation complicated by cardiogenic shock as well as the influence of the IABP on the intra-hospital mortality.

In the research carried out by Kolte et al. [14] , based on the database of patients with STEMI complicated by cardiogenic shock in the United States (US), which included around 2 million patients from 2003 to 2010, the incidence of cardiogenic shock was 7.9% and it occurred statistically significantly more frequent in patients older than 75 years as well as in women.

The average age of the patients involved in our study, the higher proportion of anterior wall myocardial infarction as well as the average age of deceased patients included in our retrospective study were in accordance with the results of studies conducted in the US, as well as the SHOCK trial registry [14, 15].

The results of our study showed a high intra-hospital mortality in the patients with acute myocardial infarction complicated by cardiogenic shock (62.6%), despite the use of primary percutaneous coronary intervention, which are significantly higher than the results of other randomized studies and registers where the intra-hospital mortality rate was 42%–48% [5–9].

The intra-hospital mortality rate, according to the results of the SHOCK II trial, was slightly lower and amounted to 40%, with the limitation that the patients with incipient cardiogenic shock were included in the study [16, 17]. In Serbia, the intra-hospital mortality rate of patients with STEMI is 7% [18].

High intra-hospital mortality in our patient sample could be explained by the strict patient selection criteria so that all patients prior to primary percutaneous coronary intervention had clear clinical signs of the acute heart failure of the Killip IV class.

There was a statistically significant difference in the mortality among the investigated groups. The lowest mortality was in the group of patients who had multi-vessel coronary disease that was suitable for complete revascularization. However, this group of patients was the most uncommon, since more frequent were patients with multi-vessel disease that was not suitable for complete myocardial revascularization (diffuse atherosclerotic disease, chronic total occlusion, massive calcification).

The results of our research showed that the average EF on echocardiography in the patients treated for acute myocardial infarction complicated by cardiogenic shock was estimated to be 36% (27%–46%) which corresponds to the results of other studies and registers. A study by Garcia-Alvarez et al. [19] carried out in Spain showed that there was a significant decrease in systolic function in the group of patients with acute myocardial infarction in cardiogenic shock who died, which was echocardiographically estimated to be 25% ± 10%. The results of the IABP-SHOCK II trial showed that among patients with acute myocardial infarction in cardiogenic shock an average EF estimated by echocardiography was 25% [16].

According to Rasoul et al. [20] and Toma et al. [21], multivessel coronary disease was present in 40% of patients with STEMI. Patients with multi-vessel coronary disease had poorer outcomes than patients with single-vessel coronary disease, and the question of whether to revascularize culprit lesion only in acute myocardial infarction or perform complete myocardial revascularization is still subject to discussion [22].

The data from the TRANSLATE ACS observational study showed that the complete revascularization in patients with STEMI and multi-vessel coronary disease was associated with the lower risk of readmission after 6 weeks as well as the lower risk of major adverse cardiovascular events after 6 weeks and 1 year [23].

The results of SHOCK study showed that, although there was no benefit after 30 days among patients with cardiogenic shock resulting from the left ventricle failure due to STEMI, the early revascularization strategy was superior to the initial medical stabilization after 6 and 12 months of follow-up, especially among patients under 75 years of age. Based on the results of the SHOCK study, early myocardial revascularization either by the percutaneous coronary intervention or by the coronary artery bypass grafting is now the class I recommendation according to the current ESC and ACC/AHA guidelines, whereby, in the state of cardiogenic shock, the revascularization of all critical stenoses or highly unstable lesions besides the culprit lesion is encouraged. The mortality rate of surgically revascularized patients was similar in this high-risk group of patients to patients who were revascularized by the percutaneous coronary intervention [2,10,11,15].

The results of our study confirmed the results of several randomized studies and registers (PRAMI, CvlPRIT) [24, 25] along with the recommendations of the European and American Society of Cardiology [10, 11]. However, they differ from the results of the recently published CULPRIT SHOCK trial on patients with acute myocardial infarction complicated by cardiogenic shock and evidence of multi-vessel coronary disease on coronarography. It showed that the risk of fatal outcome was lower in patients who underwent culprit-lesion-only pPCI compared to patients who underwent complete revascularization [26].

In our investigation, the patients with the complete myocardial revascularization and multivessel disease had significantly lower intra-hospital mortality rate than the patients with multivessel disease and culprit lesion revascularization only, and the patients with singlevessel disease.

IABP has been the most commonly used type of mechanical circulatory support for nearly five decades [27]. It improves perfusion of coronary arteries in the diastole and by decreasing afterload leads to the reduction in myocardial oxygen demand, leading to an increase in cardiac output. As for the use of IABP in cardiogenic shock caused by acute myocardial infarction, according to the results of the IABP SHOCK II study, the use of IABP did not lead to a significant reduction in a 30-day, 6-month and 12-month mortality in patients with acute myocardial infarction complicated by cardiogenic shock who underwent pPCI [16, 17]. Today, the current European as well as American STEMI guidelines, do not recommend routine use of IABP in cardiogenic shock pa-

tients (class III recommendations), except for the mechanical complications of acute myocardial infarction in order to bridge the time to surgery [10, 11].

In the period from 2007 to 2016, during which the data for our research were collected, the only available type of mechanical circulatory support at our centre was IABP. According to the results of our study, IABP was more frequently implanted in the patients with the anterior wall STEMI complicated by cardiogenic shock. This could be explained by the commonly more severe clinical state with the development of acute heart failure in the patients with the anterior wall STEMI.

Among the patients with the IABP who underwent the complete myocardial revascularization, the total intra-hospital mortality was statistically significantly lower compared to other patient groups.

The patients with TIMI 3 flow after the stent implantation on culprit lesion had lower mortality compared to the patients with TIMI flow less then 3, although there was no statistically significant difference between the groups.

Multivariate binary logistic regression using all available variables identified 4 multivariate predictors of death: age, EF, complete revascularization and TIMI flow. A prospective validation of the predictive model was planned.

Mehta et al. [28] showed in their work that when the TIMI flow was less than 3, it represented an independent predictor of mortality. In our work TIMI flow was not an independent predictor of mortality, but its impact on mortality depended on the presence of other variables.

Conclusion

STEMI complicated by cardiogenic shock is still associated with a high mortality rate. Complete myocardial revascularization, independently and in combination with IABP, significantly reduces mortality in patients with acute STEMI complicated by cardiogenic shock.

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Influence of the infiltrate density in the interstitium on the prognosis of primary glomerulonephritis

Uticaj gustine infiltrata u intersticijumu na prognozu primarnog glomerulonefritisa

Dušan Božić*†, Violeta Knežević*†, Gordana Stražmešter-Majstorović*†, Lada Petrović*†, Dejan Ćelić*†, Bojana Ljubičić*

Clinical Center of Vojvodina, *Clinic for Nephrology and Clinical Immunology, Novi Sad, Serbia; University of Novi Sad, †Faculty of Medicine, Novi Sad, Serbia

Abstract

Background/Aim. Development of inflammatory changes, fibrosis and loss of morphological structures of the interstitium have an important role in pathogenesis of primary glomerulonephritis, affecting the development, course and prognosis of the disease. The aim of this study was to determine the influence of changes in the interstitium on the prognosis of primary glomerulonephritis. **Methods.** The research included 216 patients suffering from different types of primary glumeronephritis treated at the Clinic for Nephrology and Clinical Immunology of the Clinical Center of Vojvodina, Serbia who were being monitored on average for 77.5 months. After determining on pathohistological diagnosis of the type of glomerulonephritis, renal changes in the interstitium were quantified. Numerical density in the tissue volume unit and structure of infiltrates of the interstitium were established by using the Weibel system (M42) incorporated into light microscope. Routine analyses were performed by using standard laboratory procedure. **Results.** During the research period the highest numerical density of infiltrates was verified in extracapillary glomerulonephritis (147,869 × mm^{-3}), slightly less in membranoproliferative glomerulonephritis (116,800 × mm^{-3}) and focal segmental glomerulosclerosis (96,147 × mm^{-3}), and the least being in glomerulonephritis with minimal changes (11,416 × mm^{-3}). In all types of glomerulonephritis, apart from glomerulonephritis with minimal changes, there was a significantly ($p < 0.0005$) higher numerical density and incidence of infiltrate cells in relation to the control group. By comparing the numerical density of infiltrates of all cells to the parameters of renal function, a significant ($p < 0.01$) correlation of these phenomena was established. In order to get a better insight into the speed of progression of renal failure by setting a numerical limit of the density of infiltrates < 100,000 / > 100,000 cells/mm^3, regardless of the type of glomerulonephritis, a prognostic predictor was established on the basis of which the patients with lower infiltration of the interstitium had significantly ($p < 0.005$) lower progression of renal failure. **Conclusion.** Density of infiltrates in the interstitium in primary glomerulonephritis is an important early prognostic predictor of progression of renal failure.

Key words:
glomerulonephritis; renal insufficiency; connective tissue; prognosis; histological techniques.

Apstrakt

Uvod/Cilj. Razvoj inflamatornih promena i ožiljavanja i gubitak morfoloških struktura intersticijuma zauzimaju značajno mesto u patogenezi primarnih glomerulonefritisa, što utiče na nastanak, tok i prognozu ove bolesti. Cilj istraživanja bio je da se ispita uticaj promena u intersticijumu na prognozu primarnih glomerulonefitisa. **Metode.** Ispitivanjem je bilo obuhvaćeno 216 bolesnika sa različitim tipovima primarnih glomerulonefritisa lečenih na Klinici za nefrologiju i kliničku imunologiju Kliničkog centra Vojvodine koji su praćeni prosečno 77,5 meseci. Nakon utvrđivanja patohistološke dijagnoze tipa glomerulonefritisa, kvantifikovane su promene u intersticijumu bubrega. Određivana je numerička gustina u jedinici zapremine tkiva i struktura infiltrata korišćenjem Weibel-ovog sistema (M42) inkorporisanog u svetlosni mikroskop. Rutinske analize rađene su standardnom laboratorijskom procedurom. **Rezultati:** Tokom ispitivanog perioda najveća numerička gustina infiltrata verifikovana je kod esktrakapilarnog glomerulonefritisa (147 869 × mm^{-3}), nešto manja kod membranoproliferativnog glomerulonefritisa (116 800 × mm^{-3}) i fokalnosegmentne glomeruloskleroze (96 147 × mm^{-3}), a najmanja kod glomerulonefritisa sa minimalnim promenama (11 416 × mm^{-3}). Kod svih tipova glomerulonefritisa, osim glomerulonefritisa sa minimalnim promenama, ustanovljena je značajno ($p < 0,0005$) veća numerička gustina i zastupljenost ćelija infiltrata u odnosu na kontrolnu grupu. Upoređujući

Correspondence to: Dušan Božić, Clinical Center of Vojvodina, Clinic for Nephrology and Clinical Immunology, Hajduk Veljkova street 1–7, 21 000 Novi Sad, Serbia. E-mail: dusan.bozic@mf.uns.ac.rs

numeričku gustinu infiltrata svih ćelija sa parametrima bubrežne funkcije, utvrđena je značajna ($p < 0,01$) povezanost ovih pojava. Radi boljeg uvida u brzinu progresije bubrežne insuficijencije postavljenjem numeričke granice gustine infiltrata < 100 000 / > 100 000 ćelija/mm^3 nezavisno od tipa glomerulonefritisa, ustanovljen je prognostički prediktor na osnovu kojeg su bolesnici sa manjom infiltracijom intersticijuma imali značajno ($p < 0,005$) sporiju progresiju bubrežne insuficijencije. **Zaključak.** Gustina infiltrata u intersticijumu kod primarnih glomerulonefritisa je važan, rani prognostički prediktor progresije bubrežne insuficijencije.

Ključne reči:
glomerulonefritis; bubreg, hronična insuficijencija; vezivno tkivo; prognoza; histološke tehnike.

Introduction

Primary glomerulonephritis (GN) is a significant group of diseases which can lead to chronic renal failure (CRF). The prognosis of these diseases, apart from changes on glomeruli, is also affected by inflammatory changes in interstitium which are almost always more or less present [1]. In previously conducted studies, the authors analyzed cellular infiltrates in interstitium by using monoclonal antibodies [2]. All types of primary GN reported a significant increase in the number of T lymphocytes in the interstitium from 6 to 11 times per area unit comparing to healthy kidneys in which there is a smaller number of leukocytes intraglomerularly and in the interstitium [3, 4]. The experimental models showed that cellular infiltrate always appears first perivascularly around the hilar glomerular arteriole in the first 12 hours since nephrotoxic serum has been administered, with a tendency to spread periglomerularly and only after 7 days diffusely on the interstitium [5]. Hu et al. [6] indicated that the dominant change of infiltration of the interstitium is around the glomeruli which is positively correlated with the frequency of ruptures of the Bowman capsule. It is known that infiltration of the interstitium is a prerequisite of formation of fibrosis of the interstitium and tubular atrophy whose main promoters are macrophages including a large number of mechanisms as well as the possibility for direct a macrophage-myofibroblastic change [7–9]. Therefore, the development of inflammatory changes, fibrosis and loss of morphological structure of these parts of nephrons leads to CRF progression [10].

Previous studies showed that cellular infiltrates of the lowest density can be found in postinfective GN, slightly denser in focal and diffuse mesangioproliferative GN (MzPGN foc., MzPGN dif., respectively), even denser in membranoproliferative GN (MPGN) and the densest infiltrates in extracapillary GN (RPGN). In all types of GN a positive correlation of density of cellular infiltrates with a disease prognosis was established [4]. According to the data in literature, decreasing of renal function correlates more significantly with the changes in the interstitium (infiltration, fibrosis, tubular atrophy) in comparison with the glomerular changes [11, 12]. Changes in the interstitium are an independent determinant of the development of CRF as well as primary changes on glomeruli which was confirmed in the study of Ihm [13] who have shown that hypertension is also significantly more frequent and more prevalent in the developed changes in the interstitium. Based on the abovementioned, it is important to quantify changes in the interstitium in order to provide better treatment to these patients [10].

The aim of this research was to quantify cellular infiltration in the interstitium in primary GN, compare the verified changes in relation to the control group and determine the correlation of cellular infiltration in the interstitium with the relevant parameters of the renal function.

Methods

The research involved 216 patients with different types of primary GN who were treated at the Clinic for Nephrology and Clinical Immunology of the Clinical Center of Vojvodina, Serbia. The patients were being monitored in the period of 10 years. The beginning of monitoring patients was based on establishing a histopathological diagnosis of the GN type. The end of monitoring patients was defined by the last clinical control or the diagnozed end-stage renal disease and initiation of dialysis.

In the first part of the research, demographic, biochemical parameters (concentration of urea and creatinine) and endogenous creatinine clearance were analyzed. The concentration of creatinine in the blood and urine were measured by the Jaffé method – alcaline picrate reaction. Creatinine clearance (CrCl) was calculated by using the formula: $CrCl = [U_{cr} \times 24\text{ h volume (mL)}] / [S_{cr} \times 1440\text{ (min)}]$; Note: U_{cr}-urine creatinine (μmol/L), S_{cr} - serum creatinine (μmol/L). After that, the CrCl was calculated, and the obtained value was normalized in comparison to the body surface of 1.73 m^2. The calculated values were compared to the expected values with respect to the gender and the age of the patient.

In order to get a better insight into the speed of decreasing of renal function during the research period in comparison with the GN type, we defined an average monthly decline of CrCl (mL/min) in our parients. The calculated value was the result of the difference in creatinine clearance at the begining and at the end of the research divided by the number of months of monitoring the patients.

In the second part of the research, density of cellular infiltrates of the interstitium was determined by using the quantitative method. The study included the patients who provided sufficient material for both types of microscopy and whose findings of light and immunofluorescence microscopy were compatible with regard to definitive diagnosis.

Kidney samples were taken by performing a classical percutaneous renal biopsy by using Tru-Cut needles with diameters of 1.6 and 2.0 mm. One part of the material was sent for immunofluorescence microscopy. The other part of the material was fixed in 70% alcohol, processed for hematoxylin-eosin staining and embedded in paraffin. In order to de-

termine the numerical parameters of the interstitium, the material was cut with microtome in 20 incisions at thickness of 5 μm, taking every even number incision to avoid en error of double counting the same cell, which means that 10 alternating incisions were done. Within these incisions, the kidney interstitium was being observed and interstitium infiltration was being quantitatively determined. In other words, the numerical density is a relative stereological dimension which shows how many particles there are per volume unit. In our research, for the reason of having the particle size considerably smaller than the thickness of the incision which was 5 μm, we used a thick incision and applied the Abercrombie [14] method of determining the numerical density for the thick incisions. According to this method the thickness of the incision on the side is to be observed, and the particles whose center is in the incision itself and the ones whose center is in the layer above or below the thickness of the incision, i.e., in one of the over incisions, are to be counted. In order to calculate the numerical density we used the formula according to the aforementioned author: Nv = NA / (t+D); Note: NV – numerical density, NA – the number of cells at the cross-section, t-thickness of the incision, D – the average diameter of the cells.

According to Weibel and Gomez [15], for the purpose of counting cells, a multipurpose system M42 was used and it was introduced in order to avoid a large number of cross-sections of outlines or contours of the studied structure with the test lines. Number 42 represents the number of the test lines which can be struck. The advantage of the system is transparency during counting although the mesh does not cover the entire observed field. The view of the Weibel and Gomez system on the interstitium of our patients is shown in Figure 1.

Quantifying the numerical density was performed at magnification 400, with an incorporated "mesh", i.e., a testing system, into the eyepiece (Carl Zeis GF-Pw 10×). A system incorporated into the microscopic system with a video camera was also used. In both cases there was a calibrated distance between two adjacent lines at 1.04 or 2.08 μm [16]. At least 10, but no more than 30 counts were done in all the patients. The patients who did not have at least 10 representative fields for determining the numerical density were excluded from the study. Lymphocytes, monocytes/macrophages, plasmocytes, fibrocytes and polymorphonuclear granulocytes were counted.

The control group consisted of 30 deceased persons under various accidental circumstances from the Center for Forensic Medicine, who had not been suffering from CRF. The renal tissue was analysed on the same counting conditions as in our patients. It was analyzed in 10 alternate incisions and always in 30 counts, which was possible due to sampling a larger amount of tissue.

The numerical data of the research are shown above the mean value and standard deviation, and desriptive variables above absolute and relative numbers. The data processing was performed by using the Student's *t*-test with a level of significance ($p < 0.05$), the Kaplan-Meier analysis and the Gehan-Wilcoxon test.

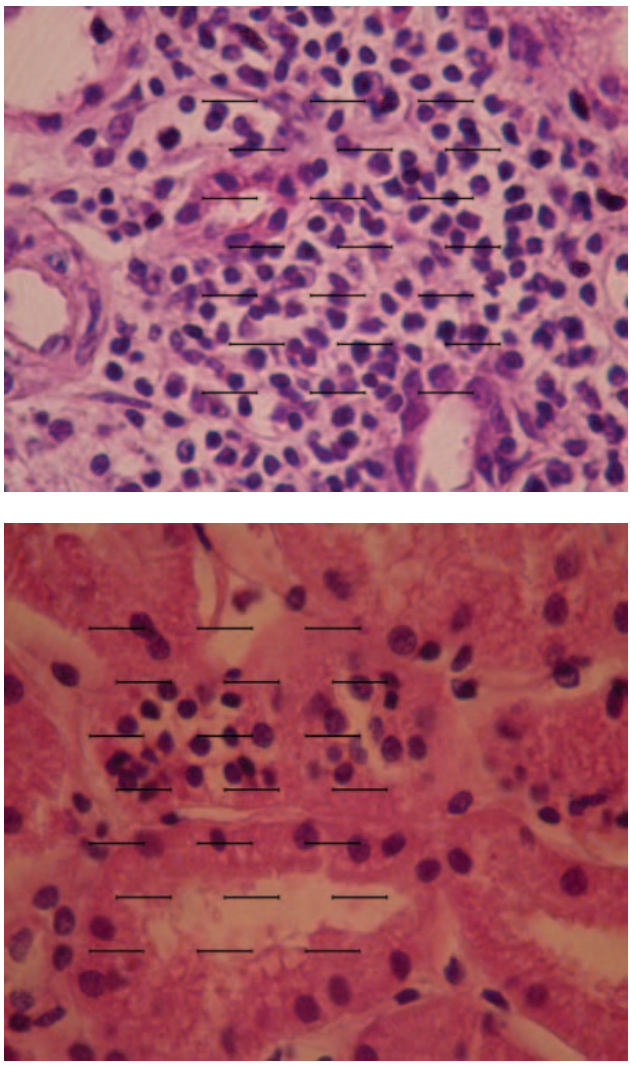

Fig. 1 – The Weibel and Gomez [15] system applied to the kidney interstitium (our material).

Results

The total number of patients involved 127 (59%) men and 89 (41%) women, the average age of 40.2 ± 11.8 and 37.9 ± 11.8 years respectively, who were diagnozed with primary GN based on the histopathological findings (Figure 2). With regard to the clinical manifestations of GN, the nephrotic syndrome was the most prevalent in membranous glomerulonephritis (MGN) 88.9% and glomerulonephritis with minimal changes (MCGN), 87.5%; persistent microscopic haematuria (with or without proteinuria) in IgA nephropathy (IgA), 81.25% and MzPGN foc., 80%; chronic nephritis syndrome in focal segmental glomerulosclerosis (FSSH), 33.3%; and the rapidly progressive nephritis syndrome was most frequent in RPGN, 85.71%.

The longest monitoring period was performed in the IgA patients whereas the shortest monitoring period was in the RPGN patients (109.75 ± 38.34 and 18.6 ± 19.17 months, respectively).

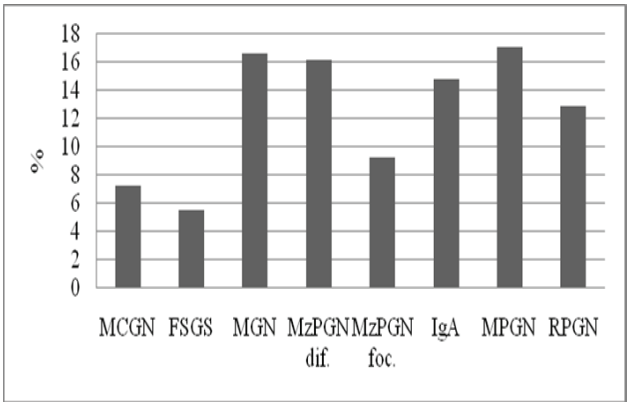

Fig. 2 – Frequency of primary glomerulonephritis.

MCGN – minimal change glomerulonephritis; FSGS – focal segmental glomerulosclerosis; MGN – membraneus glomerulonephritis; MzPGNdif – diffuse mesangioproliferative glomerulonephritis; MzPGNfoc – focal mesangioproliferative glomerulonephritis; IgA – IgA nephropathy; MPGN – membranoproliferative glomerulonephritis; RPGN – rapidly progressive (crescent) glomerulonephritis.

The numerical density and infiltrate structure of the interstitium were determined and a significantly ($p < 0.001$) higher total density of infiltrates as well as individual cells of infiltrates were established in the patients comparing to the control group. The infiltrate structure of the patients suffering from GN abounded with lymphocytes, followed by the number of monocyte while less prevalent were other types of cells (fibrocytes, plasmocytes and polymorphonuclear granulocytes). However, in the patients with healthy kidneys, apart from significantly less density of infiltrates, the incidence of lymphocytes and monocytes was almost equal (Table 1). In the patients diagnozed with a worse type of GN (RPGN, MPGN, FSSH) a higher density of infiltrates comparing to other types of GN was verified (Table 2). A significant ($p < 0.0005$) difference among the patients regarding the GN type and the control group for the numerical density and structure of infiltrates in all GN, apart from MCGN, was established. In order to have a more transparent view of the results in Table 3 only the values of the structure and density of cellular infiltrate in RPGN, MCGN and the control group are shown.

Table 1

The total numerical density and structure of interstitial infiltrate in the patients compared to the control group

Structure of infiltrate	Patients (n = 216) mean ± SD	Control group (n = 30) mean ± SD	*p*
Lymphocytes	57682.3 ± 10816.0	5955.7 ± 2652.5	< 0.001
Monocytes	18783.6 ± 11360.9	5132.20±1447.9	< 0.001
Fibrocytes	3246.71 ± 2388.8	282.03 ± 119.1	< 0.001
Plazmocytes	1886.0 ± 2249.7	183.33 ± 85.1	< 0.001
PMN	1384.9 ± 879.4	239.50 ± 73.6	< 0.001

All values are meaning cell density/mm^3; PMN – polymorphonuclear granulocytes; SD – standard deviation.

Table 2

The numerical density of cells in the interstitium in relation to the type of glomerulonephritis

Type of GN	Structure infiltrate (mm^3)					
	Lymphocytes mean ± SD	Monocytes mean ± SD	Fibrocytes mean ± SD	Plazmocytes mean ± SD	PMN mean ± SD	Total density mean ± SD
MCGN	8,049.9 ± 2,901.7	2,567.3 ± 936.6	365.1 ± 122.2	254.3 ± 92.4	180.3 ± 71.8	11,416.9 ± 4,095.7
FSGS	59,990.8 ± 10,816.0	29,141.8 ± 4,804.8	3,847.3 ± 657.1	1,872.2 ± 408.0	1,295.2 ± 290.4	96,147.3 ± 16,238.8
MGN	33,012.4 ± 13,170.3	10,930.6 ± 4,498.3	1,856.1 ± 1,008.3	1,016.8 ± 368.4	772.2 ± 354.3	47,588.1 ± 19,259.0
MzPGd.	66,571.2 ± 39,194.6	20,905.9 ± 11,692.0	3,890.4 ± 3,229.0	2,065.1 ± 1,211.9	1,475.9 ± 889.6	94,908.4 ± 55,822.9
MzPGf.	39,882.9 ± 24,497.7	3,068.1 ± 7,545.7	1,939.5 ± 1,211.6	1,226.0 ± 727.9	1,255.5 ± 1,178.4	57,372.0 ± 34,576.7
IgA	42,204.5 ± 15,807.0	13,319.6 ± 5,222.3	2,275.1 ± 1,112.5	1,319.3 ± 702.5	1,620.0 ± 824.6	60,738.4 ± 23,243.1
MPGN	81,813.4 ± 26,290.8	26,480.9 ± 8,269.0	4,635.5 ± 1,884.9	2,214.8 ± 605.5	1,655.5 ± 543.7	116,800.1 ± 37,032.5
RPGN	104,176.6 ± 27,070.3	31,210.5 ± 8,028.7	5,828.2 ± 1,865.8	4,402.6 ± 5,170.1	2,251.9 ± 690.5	147,869.9 ± 35,998.4
C. G.	5,955.7 ± 2,652.5	5,132.2 ± 1,447.9	282.0 ± 119.1	183.3 ± 85.1	239.5 ± 73.5	11,792.7 ± 4,154.3

GN –glomerulonephritis; MCGN – minimal change glomerulonephritis; FSGS – focal segmental glomerulosclerosis; MGN – membranous glomerulonephritis; MzPGNd. – diffuse mesangioproliferative glomerulonephritis; MzPGNf. – focal mesangioproliferative glomerulonephritis; IgA – IgA nephropathy; MPGN –membranoproliferative glomerulonephritis; RPGN – rapidly progressive (crescent) glomerulonephritis; C.G. – control group; PMN – polymorphonuclear granulocytes.
All values are meaning cell density/mm^3.
SD – standard deviation.

Table 3

The numerical density and structure of interstitial infiltrate in MCGN and RPGN compared to the control group

Structure of infiltrate (mm^3)	MCGN/control group		RPGN/control group	
	mean ± SD	*p*	mean ± SD	*p*
Ly.	8,049.9 ± 2,901.7/5,955.7 ± 2,652.5	0.0171	104,176.6 ± 27,070.3/5,955.7 ± 2,652.5	< 0.0005
Mon.	2,567.3 ± 936.6/5,132.0 ± 1,447.9	< 0.0005	31,210.5 ± 8,028.7/5,132.2 ± 1,447.9	< 0.0005
Fibr.	365.1 ± 122.2/282.0 ± 119.1	0.0307	5,828.2 ± 1,865.8/282.03 ± 119.1	< 0.0005
Plaz.	254.4 ± 92.4/183.3 ± 85.1	0.0121	4,402.6 ± 5,170.1/183.33 ± 85.1	< 0.0005
PMN	180.3 ± 71.8/239.5 ± 73.5	0.0119	2,251.9 ± 690.5/239.5 ± 73.5	< 0.0005
T. D.	11,416.9 ± 4,095.7/11,792.73 ± 4,154.3	0.7703	147,869.9 ± 35,998.4 / 11,792.73 ± 4,154.3	< 0.0005

MCGN – minimal change glomerulonephritis; RPGN – rapidly progressive (crescent) glomerulonephritis; Ly. – lymphocytes; Mon. – monocytes; Fibr. – fibrocytes; Plaz. – plazmocytes; PMN – polymorphonuclear granulocytes; T.D. – total density. All values are meaning cell density/mm^3.
SD – standard deviation.
***p* < 0.05.**

Table 4 shows an average monthly decline of creatinine clearance as a parameter of deterioration of renal function in comparison with the GN type. The difference values of serum creatinine and CrCl at the beginning and at the end was 130 mmol/L and 21.3 mL/min, respectively in all patients. By comparing the numerical density of infiltrates of all cells to the creatinine increase, the CrCl decline and the average monthly decline of CrCl, a significant correlation between these phenomena was determined (r = 0.4484, r = 0.2244, r = 0.5055, respectively; $p < 0.01$).

In the final part of the research a numerical limit of the infiltrate density < 100.000 / > 100.000 cells/mm^3 was set regardless of the GN type. Comparing these two groups of patients by using the Kaplan-Meier analysis and comparing the two curves by using the Gehan-Wilcoxon test, a prognostic predictor according to which the patients suffering from less infiltration of the interstitium had a significantly ($p < 0.005$) slower progression of CRF (Figure 3) was established.

Table 4

The average monthly decline of creatinine clearance (CrCL)

Type of glomerulonephritis	CrCl (mL/min) mean ± SD
Focal segmental glomerulosclerosis	0.017 ± 0.042
Minimal change glomerulonephritis	0.734 ± 0.228
Membranous glomerulonephritis	0.376 ± 0.453
Diffuse mesangioproliferative glomerulonephritis	0.189 ± 0.220
Focal mesangioproliferative glomerulonephritis	0.102 ± 0.164
IgA nephropathy	0.227 ± 0.282
Membranoproliferative glomerulonephritis	0.547 ± 0.383
Extracapillary glomerulonephritis	0.765 ± 0.669

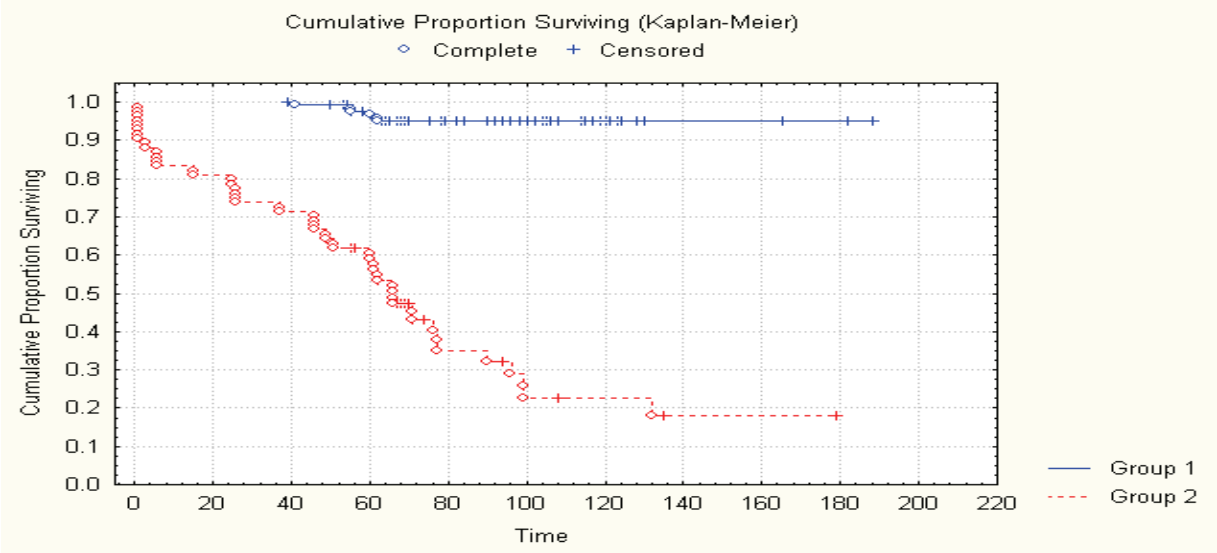

Fig. 3 – Renal function in relation to the numerical density of interstitial infiltration.
Group 1 – numerical density < 100,000 cells/mm^3; Group 2 – numerical density > 100,000 cells/mm^3.
***p* < 0.005 (Gehan-Wilcoxon test).**

Discussion

Glomerular and tubulointerstitial lesions cause a certain level of damage of renal function and affect the outcome of the disease in patients suffering from primary or secondary GN [10].

In our study, the patients we were diagnozed with all types of primary GN manifested in different clinical syndromes during different periods of monitoring, which matches the results in several published studies. Considering the fact that the clinical work revealed histopathological findings of kidney biopsy without glomerular sclerosis, and that the clinical signs of CRF were already present in the patients and vice versa, and considering the fact that the available literature had not provided us with the knowledge of measured numerical density of infiltrates in primary GN, we think ,as well, it is necessary for an early prognosis of the disease to examine the changes in the interstitium over several years of monitoring.

By using a stereological method of determining numerical density of cellular infiltrates, the changes in the interstitium were verified and quantified. A multipurpose system according to Weibel and Gomez [15] was used and the cell density per volume unit of the interstitium tissue was determined [17, 18]. Apart from the total cell infiltration in the interstitium, the types of cells which infiltrate contains were also analyzed individually. The results obtained were compared to the finding of the same parameters in the interstitium in the control group because their minimal cell infiltrates were also verified. Our patients had significantly higher numerical density and infiltrate structure in comparison with the control group, as expected. In primary GN, the cellular infiltrate abounded with lymphocytes, which contributed almost 2/3 to the total number of cells, followed by a three times smaller number of monocytes, whereas other cells were less prevalent. Several authors indicated that the presence of a large number of lymphocytes and monocytes/macrophages was responsible for the occurrence of changes in the interstitium in primary GN, which was confirmed in this research as well [1, 4, 19]. Unlike our results, Schena et al. [20] showed a slightly higher density of polymorphonuclear granulocytes in IgA in relation to other types of GN. However, the healthy kidneys had a ratio between lymphocytes and monocytes almost 1:1, which caused the infiltrate structure to be completely different.

The highest numerical cell density was in the types of GN in which the expected prognosis was worse (RPGN, FSSH i MPGN). The average values of the numerical density were from 11,417/mm^3 in MCGN to 147,870/mm^3 in RPGN, unlike the healthy kidneys whose value was 11,792/mm^3. The total number of cells in the interstitium in MCGN was slightly smaller than in the healthy kidneys. Comparing to the control group, the numerical density and infiltrate structure in all types of GN, apart from MCGN, were significantly higher, which is in accordance with the results presented in one study, although different techniques of cell quantification in the interstitium were used [21].

While examining the changes in the interstitium in primary GN, some authors used different methods of quantifying changes. Hooke et al. [3] quantified cells in the interstitium by using the monoclonal antibodies typical of certain cells. Although the way is accurate, croos-sections are possible in only one geometrical plane, thus the results were shown as a number of verified cells per square millimeter. Li et al. [22] used a similar technique to prove the distinct changes in the IgA patients who had more rapid progression of CRF. The given studies reported similar results to our results.

The authors of previous studies indicated that infiltration of the interstitium, density and the number of cells correlate significantly with deterioration of renal function, even more effectively than the changes in glomeruli [11, 12]. At the beginning of the research a decrease of renal function in most of our patients was verified, therefore they were monitored in different periods of time depending on the GN type and the course of the disease,clinical and laboratory parameters. Through relevant indicators of renal function at the beginning and the end of the research a corresponding stage of CRF was verified. However, for the purpose of clearer understanding of the decline speed of renal function during the examining period related to the GN type, we determined an average monthly decline of creatinine clearance, whose obtained values were the lowest in MCGN, and the highest in RPGN (0.017 and 0.765 mL/min/month, respectively).

We established a significant correlation among the increase of creatinine, the decline of CrCl, the average monthly decline of CrCl and the numerical cell density in the interstitium, thus showing that denser cellular infiltrates appeared considerably more frequently in the patients who had more rapid progression of CRF. Similar results are revealed in some studies [10, 23].

The research showed that infiltration of the interstitium had an impact on renal function and therefore on the GN prognosis. In order to get a better insight into the speed of the CRF progression, by setting a numerical limit of the infiltrate density < 100,000 / > 100,000 cells/mm^3, regardless of the GN type, a prognostic predictor was set according to which the patients suffering from a lower infiltration of the interstitium had a significantly slower progression of CRF. According to the predictor, the numerical density higher than 100,000/mm^3 indicated more rapid progression of CRF towards the end stage renal disease and simultaneous deterioration of the pathohistological findings through verified changes characteristic of fibrosis, which was proven in several studies [24]. Therefore, over the last few years, a considerable effort was made to discover appropriate inflammation inhibitors, primarily endogenous, which would decrease infiltration of the interstitium, modify the role of proximal tubular cells to infiltration and fibrosis and slow down the progression of CRF [25–27].

Conclusion

Our research established that the quantitatively determined changes in the interstitium are an important early predictor of the GN prognosis, therefore, by using the prognostic predictor, they could represent an additional criterion when deciding on the treatment of patients, taking into con-

sideration not only the GN type as a basic parameter for determining a treatment protocol. The given method of determining the numerical density requires use of light microscope with few adaptations, which makes it available and inexpensive.

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Prevalence and risk factors of *Chlamydia trachomatis* genital infection among military personnel of the Armed Forces of Serbia: a cross-sectional study

Prevalencija i faktori rizika od genitalne infekcije koju uzrokuje *Chlamydia trachomatis* među pripadnicima Vojske Srbije: studija preseka

Željko Jadranin*, Elizabeta Ristanović[†‡], Sonja Atanasievska[†], Gordana Dedić[‡§], Sandra Šipetić-Grujičić[‖], Dubravko Bokonjić[¶], Michael Grillo, Jovan Mladenović*, Vladimir Bančević[‡††], Branko Košević[‡††], Vesna Šuljagić[‡‡‡]**

Military Medical Academy, *Institute of Epidemiology, [†]Institute of Microbiology, [§]Clinic for Psychiatry, [¶]National Poison Control Center, [††]Clinic for Urology, [‡‡]Department for Prevention and Control of Nasocomial Infections, Belgrade, Serbia; University of Defence, [‡]Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, [‖]Institute of Epidemiology, Belgrade, Serbia; **Department of Defence HIV/AIDS Prevention Program, United State of America

Abstract

Background/Aim. *Chlamydia trachomatis* infection (CTI) is an increasing public health problem worldwide and is the most frequent sexually transmitted infection. Studies conducted in many armed forces worldwide showed that CTI is common within military population and generate significant healthcare costs. The aim of this study was to estimate the prevalence of CTI among members of the Serbian Armed Forces (SAF) and to determine risk factors for this infection. **Methods.** The study was designed as a cross-sectional survey and consisted of completing a questionnaire and chlamydial testing. The questionnaire was divided into two question groups: one was about demographic/service related characteristics and the other was about behavioral patterns/habits. *Chlamydia trachomatis* real time polymerase chain reaction (Real-TM PCR) was used for detection of pathogen genome specific sequence in the male urethral swabs and in the female endocervical swabs. All data collected were used to compare military personnel with and without CTI. Risk factors independently associated with CTI were identified by the stepwise multivariate logistic regression analysis (MLRA) of variables selected by the univariate logistic regression analysis (ULRA), with a limit for entering and removing variables from the model at 0.05. **Results.** The overall prevalence of CTI was 55 of 356 respondents (15.4%; 95% CI 0.5–2.7%). The ULRA identified that CTI was significantly associated with several characteristics: number of sexual partners during previous year ($p = 0.008$), knowledge about symptoms of CTI ($p = 0.035$), tattooing ($p = 0.035$) and married or in stable relationship ($p = 0.022$). The MLRA revealed that number of different sexual partners during last year was independent risk factor of CTI ($p = 0.026$; OR : 0.344; 95% CI: 0.13–0.88). **Conclusion.** CTI is significant problem in male and female military personnel in the SAF. The number of different sexual partners during previous year was independently associated with CTI. These finding indicates that screening for CTI should be undertaken in the SAF, to reduce rates of CTI in the SAF and to prevent morbidity due to this infection.

Key words:
chlamydia trachomatis; reproductive tract infections; military personnel; risk factors; prevalence; serbia.

Apstrakt

Uvod/Cilj. Infekcija sa *Chlamydia trachomatis* (ICT) je rastući javno zdravstveni problem širom sveta i predstavlja najučestaliju polno prenosivu infekciju. Istraživanja u mnogim vojskama su pokazala da je ova infekcija česta i među pripadnicima vojne populacije i da čini značajan udeo u troškovima njihovog lečenja. Cilj ove studije je bio da istraži učestalost ICT među pripadnicima Vojske Srbije (VS), kao i da definiše faktore rizika od ICT. **Metode.** Studija je koncipirana kao studija pre-

Correspondence to: Željko Jadranin, Military Medical Academy, Sector for Preventive Medicine, Institute of Epidemiology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: zeljko.jadranin@yahoo.com

seka. Ispitanici su popunjavali epidemiološki upitnik i bili su testirani na prisustvo ICT. Upitnik je bio podeljen na dve grupe pitanja: o demografskim podacima i podacima u vezi sa vojnom službom, kao i pitanja o ponašanju i navikama ispitanika. Za dokazivanje ICT u uretralnom brisu muškaraca, odnosno endocervikalnom brisu žena korišćen je *real-time polymerase chain reaction* (Real-TM PCR). Svi prikupljeni podaci su korišćeni za poređenje ispitanika sa i bez ICT. Faktori rizika nezavisno povezani sa ICT su identifikovani multivarijantnom logističkom regresionom analizom (MLRA) varijabli identifikovanih u univarijantnoj logističkoj regresionoj analizi (ULRA) (korišćene varijable sa $p \leq 0,05$). **Rezultati.** Ukupna prevalencija ICT u ispitivanoj populaciji je iznosila 55 od 356 ispitanika (15,4%; 95% CI 0,5–2,7%). ULRA je pokazala da su sa ovom infekcijom statistički značajno povezani: broj različitih seksualnih partnera u prethodnih godinu dana ($p = 0,008$), znanje o simptomima ICT ($p = 0,35$), tetoviranje ($p = 0,035$) i brak ili stabilna veza ($p = 0,022$). MLRA je otkrila da je broj različitih seksualnih partnera u prethodnih godinu dana nezavisni factor rizika od ICT ($p = 0,026$; OR: 0,344; 95% CI: 0,13–0,88). **Zaključak.** ICT je značajan zdravstveni problem za pripadnike VS oba pola. Broj različitih seksualnih partnera tokom prethodnih godinu dana je nezavisno povezan sa ovom infekcijom. Rezultati studije ukazuju na opravdanost uvođenja periodičnog skrininga na ICT kod svih pripadnika VS, što bi dovelo do smanjivanja učestalosti ove infekcije, kao i preveniranja njenih komplikacija.

Ključne reči:
chlamydia trachomatis; polni organi, infekcije; kadar, vojni; faktori rizika; prevalenca; srbija.

Introduction

Chlamydia trachomatis infection (CTI) is an increasing public health problem worldwide and the most frequent sexually transmitted infection (STI) [1, 2]. According to the data of the World Health Organization (WHO), there are about 131 million new infections yearly [1]. CTI is common both in men and women and with the highest rates among 20–24 years olds, followed by 15–19 years olds [2, 3].

The main mode of transmission of CTI is through sexual contact, including vaginal, anal and oral sex. In addition, CTI can be transmitted from mother to child during pregnancy and childbirth [2].

CTI can be detected in urogenital system, but a site of infection could also be the eye, pharynx and rectum [4].

Urogenital infection in both sexes most commonly presents with urethritis, characterized by dysuria and urethral discharge. Left untreated, it can lead to serious complications such as cervicitis, chronic pain, ectopic pregnancy and infertility in women as well as epididymitis, prostatitis and proctitis in men [5, 6].

In typical cases, adult chlamydial eye infection manifests as follicular conjunctivitis, characterized by mucopurulent discharge, redness and foreign body sensation. About 80% of these patients have concurrent urogenital infections [7]. Neonatal conjunctivitis develops in 20%–50% of babies born to mothers with chlamydial cervical infection and can lead to focal corneal neovascularisation, scarring, pannus formation, and chronic conjunctivitis [8].

Pharyngeal CTI can cause pharyngitis and lymphadenitis [9]. Rectal CTI can cause rectal pain, bleeding and discharge as well as proctitis [4]. Pharyngeal and rectal infections are most common among women and men who have sex with men [4].

CTI is curable with effective single-dose regimens of antibiotics [1]. However, a major concern with CTI is that most patients are asymptomatic. Some studies estimated that only about 10% of men and 5%–30% of women with laboratory-confirmed CTI develop symptoms [10, 11]. Moreover, in absence of symptoms the majority of extragenital infections are undiagnosed, untreated, and, as a result, remain important reservoir for further CTI transmission [4].

The Center for Disease Control and Prevention (CDC) recommends annual screening for CTI in all sexually active women younger than 25 years. Women older than 25 years as well as all sexually active men, should be screened if they are at a risk (current STI, new or multiple sex partners, inconsistent condom use, drug use, commercial sex work, and/or high community prevalence of STIs, etc.) [5].

Studies conducted in the Armed Forces of Poland [12], Estonia [13], Brazil [14], Israel [15], Slovenia [16] and the United States of America [17], shown that CTI is common within military population. High rates of CTI among military population generate significant healthcare costs in the armies [18].

Until now, there has been no study about prevalence and risk factors (RF) for CTI among members of the Serbian Armed Forces (SAF). The aim of this study was to estimate the prevalence of among members of the the SAF and to determine RF for this infection.

Methods

Study design

The study was designed as a cross-sectional survey and performed during January–June 2016. Participation in the study was voluntary and all participants gave written consent. The study consisted of completing the questionnaire and chlamydial testing. The Research Ethics Board of the Military Medical Academy (MMA) Belgrade, Serbia approved the research protocol.

Study population

The study was undertaken in eight barracks across Serbia: Sombor, Belgrade, Niš, Valjevo, Vranje, Pančevo, Zaječar and Požarevac. Approximately, 1,500 soldiers who were present in the barracks were given an educational briefing about CTI and after that they were invited to enroll in

the study. A total of 356 members of the SAF volunteered to participate in the study. Exclusion criteria were subjects that had been taking antibiotics within six weeks prior to chlamydial testing, urinating two hours before sample collection and currently diagnosed with CTI.

Data collection

The participants completed a self-administered questionnaire. The questionnaire was divided into two parts. The first part consisted of demographic and service related characteristics: gender, age, marital status, military rank, service/branch, years of service in the army and participation in peacekeeping missions. The second part of questionnaire asked questions about behavioral patterns and habits: age at first sexual intercourse, number of sexual partners during last year, lifetime number of sexual partners, multiple sexual partners (more than one sexual partners at the same period of time), sexual intercourse at first meeting, sexual workers as sexual partners, frequency of condom use, habits related to alcohol consumption, smoking, tattooing, knowledge about symptoms of CTI and regularity of gynecological examinations for the female participants. All data collected were used to compare military personnel with and without CTI.

Laboratory procedures and treatment

Chlamydia trachomatis real-time polymerase chain reaction (Real-TM PCR) kit (Sacace Biotechnologies) was used for detection of pathogen genome specific sequence in the male urethral swabs as well as in the female endocervical swabs. This test is based on the real-time hybridization-fluorescence detection (exquisitely sensitive and highly specific). CTI positive military personnel were given antibiotics and were retesting for 6 weeks after the therapy. Also, they were advised that all their sexual partners should be treated from CTI.

Statistical analysis

Data analyses were performed with the SPSS, version 18. The prevalence was defined as a number of the CTI positive participants per 100 tested. To test the statistical significance of the difference, the χ^2-test was used. The odds ratio (OR) and its respective 95% confidence interval (CI) for each variable were calculated. The RF independently associated with CTI were identified by the stepwise multivariate logistic regression analysis (MLRA) of the selected variables by the univariate logistic regression analysis (ULRA), with a limit for entering and removing variables from the model at 0.05.

Results

Demographic and service related information

A total of 356 respondents participated in the study, with 306 (85.9%) male participants and 50 (14.1%) females. Overall, the study population median age was 30.9 years (range 19–59 years). Majority of the study participants, [230 (64.6%)] were married or in long-term, stable relationships while others were single. The participants had different ranks and most often they were contract soldiers [117 (32.9%)], or officers [79 (22.2%)]. More than a half of the respondents [187 (52.5%)] had 5 or fewer years in service, while 118 (33.1%) were in the military service more than 10 years. The distribution of their service/branches were as follows: 199 (55.9%) of them were in the Army, 137 (38.5%) in Logistics, 73 (20.5%) and 20 (5.6%) in the Air Force. Only 12 (3.4%) respondents participated in the peacekeeping operations.

Prevalence of the CTI

The overall prevalence of CTI was 55 of 356 resodents (15.4%; 95% CI 0.5-2.7%). *Chlamydia trachomatis* specific genome sequence was detected in the 7 of 50 endocervical swabs (14%) and in 48 (15.7%) of 306 urethral swabs.

Risk factors

The ULRA identified that CTI was significantly associated with several characteristics: number of sexual partners during previous year ($p = 0.008$), knowledge about symptoms of CTI ($p = 0.035$), tattooing ($p = 0.035$) and being married or in stable relationship ($p = 0.022$).

Other demographic and service related characteristics (Table 1) as well as behavioral patterns and habits (Table 2) were not significantly associated with CTI.

After entering the significant variables into the MLRA, a number of different sexual partners during last year significantly interact with other selected parameters and was an independent RF of CTI (Table 3).

A characteristic that was examined only for female participants in the study, was regularity of the gynecological examinations. Among 43 CTI negative female participants 42 (97.7%) had regular gynecological examinations, at least once per year, while 1 (2.3%) CTI negative female participant had the gynecological examination only occasionally. Among 7 CTI positive female participants, 4 (57.1%) had the regular gynecological examinations and 3 (42.9%) had them occasionally ($p = 0.007$; OR: 0.032; 95%CI: 0.003–0.381).

Discussion

We found a prevalence rate of CTI of 15.4% among the members of the SAF. This CTI prevalence is higher than previously reported in the most of other studies conducted within the military population, noting the prevalence ranging from 2.5%–9.5% [13, 15, 19, 20]. Our results were similar to a large survey among female military recruits conducted in the United States of America in 1997. This survey showed that the CTI prevalence was 10% –15% of recruits from New Jersey, North Carolina, Kentucky, Texas, Oklahoma and Arkansas and more than 15% of recruits from South Carolina, Georgia, Alabama, Louisiana and Mississippi [21].

Table 1

Demographic and service related characteristics associated with *chlamidia trachomatis* infections (CTI)

Characteristics	CTI negative n = 301 n (%)	CTI positive n = 55 n (%)	*p*	OR	95% CI	
					lower	upper
Sex						
male	258 (85.7)	48 (87.3)	0.760	1.143	0.485	2.690
female	43 (14.3)	7 (12.7)				
Age, (years)						
≤ 20	13 (4.3)	2 (3.6)	0.429	0.821	0.146	4.606
21–30	141 (46.8)	32 (58.2)	0.822	1.210	0.467	3.138
31–40	115 (38.3)	15 (27.3)	0.694	0.696	0.250	1.938
≥ 41	32 (10.6)	6 (10.9)	0.488			
Stable relationship	202 (67.1)	28 (50.9)	0.022	0.508	0.284	0.908
Rank						
officer	66 (21.9)	13 (23.6)	0.571	1.379	0.454	4.183
NCOs	58 (19.4)	5 (9.1)	0.449	0.603	0.163	2.233
contract soldier	95 (31.6)	22 (40.0)	0.365	1.621	0.570	4.612
civilian serviceman	20 (6.7)	2 (3.6)	0.686	0.700	0.124	3.946
conscript soldier	47 (15.6)	8 (14.6)	0.243	2.074	0.609	7.060
cadets	35 (11.6)	5 (9.1)				
Service/branch						
army	167 (55.5)	32 (58.2)	0.853	1.058	0.581	1.927
air Force	18 (6.0)	2 (3.6)	0.533	0.614	0.132	2.843
logistics	116 (38.5)	21 (38.2)				
Years in service						
≤ 5	159 (52.8)	28 (50.9)	0.581	1.209	0.616	2.373
6–10	39 (13.0)	12 (21.8)	0.082	2.113	0.909	4.913
≥ 11	103 (34.2)	15 (27.3)				
Peacekeeping missions	9 (3.0)	3 (5.5)	0.359	1.872	0.490	7.145

OR – odds ratio; CI – confidence interval.

Table 2

Sexual behavior patterns and habits associated with *chlamidia trachomatis* infections (CTI)

Sexual behaviour	CT negative (n = 301) n (%)	CT positive (n = 55) n (%)	*p*	OR	95% CI	
					lower	upper
Age at first sexual intercourse (years)						
≤ 15	32 (10.6)	11 (20.4)	0.102	1.765	0.732	4.256
16–18	192 (63.8)	28 (51.8)	0.206	0.749	0.379	1.479
≥ 19	78 (25.6)	15 (27.8)	0.404			
No of sexual partners during last year						
≤ 1	181 (59.8)	25 (46.3)	0.008	0.250	0.104	0.602
2–5	103 (34.3)	19 (35.2)	0.002	0.332	0.133	0.829
≥ 6	18 (5.9)	10 (18.5)	0.018			
No of lifetime sexual partners						
≤ 1	18 (5.6)	3 (5.6)	0.350	0.667	0.176	2.528
2–10	165 (54.8)	27 (50.0)	0.551	0.618	0.320	1.196
11–20	51 (16.9)	6 (11.1)	0.153	0.444	0.444	1.199
≥ 21	68 (22.7)	18 (33.3)	0.109			
Multiple sexual partners	156 (51.8)	27 (50.0)	0.709	0.896	0.504	1.593
Sexual intercourse at first meeting	135 (44.8)	29 (52.7)	0.282	1.372	0.771	2.440
Sexual workers as sexual partner	23 (7.6)	6 (10.9)	0.418	1.480	0.573	3.821
Condom use			0.626			
regularly	64 (21.3)	10 (18.2)	0.399	0.699	0.304	1.606
occasionally	152 (50.5)	26 (47.3)	0.419	0.765	0.400	1.463
never	85 (28.2)	19 (34.5)				
Alcohol consumption	269 (86.0)	50 (90.9)	0.331	1.622	0.611	4.301
Smoking	111 (36.8)	16 (29.1)	0.269	0.702	0.375	1.315
Tattooing	38 (12.6)	13 (23.6)	0.035	2.142	1.054	4.353
Knowledge about symptoms of CTI						
yes	22 (7.3)	9 (16.4)	0.035	3.273	1.085	9.871
no	223 (74.1)	39 (70.9)	0.442	1.399	0.594	3.294
don't know	56 (18.6)	7 (12.7)				

OR – odds ratio; CI – confidence interval.

Table 3

Multivariant logistic regression

Number of sexual partners during last year	Wald	*p*	OR	95% CI	
				lower	upper
≤ 1	4.928	0.026	0.344	0.134	0.883
2–5	5.232	0.022	0.335	0.131	0.855
≥ 6	1.796	0.180	0.447		

OR – odds ratio; CI – confidence interval.

Our study shows that the prevalence among female participants was 14% and among male was 15.7%. Although the data from the WHO [1] and CDC [5] as well as from some studies the military populations [17, 22–24] indicate that CTI is more common among females, our investigation showed different results. One possibility could be due to the fact that only 14% of our study population were females. Also, the prevalence of CTI among males is underestimated since there was no regular screening on CTI among males worldwide. With the increased availability of the urine testing, men are increasingly being tested for CTI in the last several years [20]. During 2010–2014, the CTI in men increased for 22%, compared with 6% increase in women during this period [6].

The studies conducted in the military populations showed considerable variations in determining the RF for CTI. For example, a study conducted among female military recruits in the US Army [21] showed that the young age was associated with CTI both in the ULRA and MLRA. Similar results are also found in a number of other studies [17, 18, 22]. In our study, the age was not significant RF, but still had the highest CTI prevalence in the population younger than 30 years.

The ULRA identified that CTI was significantly associated with several characteristics of our respondents: number of sexual partners during the previous year, knowledge about symptoms of CTI, tattooing, being married or in a stable relationship.

The respondents who were married, or in a stable relationship, had significantly lower risk for CTI than those who were single ($p = 0.022$, OR: 0.508, 95% CI: 0.284–0.908). Similar results were found in the studies conducted among military populations by Jordan et al. [17] and Barnett and Brundage [25]. Respondents who were in a stable relationship had same sexual partner for a long period of time and were not at risk of acquiring CTI. This explanation is even more reasonable when we know that other significant RF associated with CTI was number of different sexual partners during last year (Table 2). A protective factor for acquiring CTI was lower number of sexual partners. Compared with those who had more than five sexual partners during last year, those with one sexual partner had OR: 0.250; 95% CI: 0.104-0.602.

The MLRA for independently significant RF also showed that number of different sexual partners during the previous year was still stable and significantly interacted with other selected parameters.

A significant connection also existed between CTI and tattooing (12.6% *vs* 23.6%; $p = 0.035$; OR: 2.142; 95% CI: 1.054–4.353). Since tattooing is associated with the transmission of CTI, a possible explanation may be a result of behavior. Tattooing is allowed for members of the SAF, but it is not common. Therefore, we can assume that among military population in Serbia, the tattooed people have tendency to express other forms of risky behavior. A possible reason for this correlation between CTI and tattooing could be that reporting tattoo is culturally acceptable, but could indicate a more riskier form of behavior.

Those who had knowledge about CTI symptoms were significantly more frequent in the group of respondents with CTI (16.4% *vs* 7.3%; $p = 0.035$, OR: 3.273, 95% CI: 1.085–9.871). We did not find any studies conducted in the military populations that examine the connection between knowledge about CTI symptoms and the risk of acquiring it, but there are several studies that show that health education is important in the prevention of HIV/AIDS as well as other STIs [26–28].

Finally, the women who regularly had the gynecological exams were in a lower risk for CTI than the women who did those exams occasionally (97.7% *vs* 57.1%; $p = 0.007$; OR: 0.032; 95% CI 0.003–0.381). Based on these data, it seems that the regular gynecological exams are protective factors against acquiring CTI. Of course, that is not case, and probable explanation is that women who regularly visit their gynecologist have more chance that CTI will be recognized and diagnosed, or less chance to accidentally find out their CTI status.

Some studies show that failures to use condoms were significantly associated with CTI [14, 24]. In our study, as well as in a study among the Male College Reserve Officer Training Corp Cadets [19], irregular condom use was not a significant RF for CTI. This data could not be interpreted alone (for those who have only one sexual partner, the irregular condom use is not a RF for any STIs) and they still could indicate that self-reported sexual-risk histories are not always valid.

When compared to different services/branches in the SAF, the highest CTI prevalence was found in the Army (16.1%) and the lowest among members of the Air Force (10%). This is in accordance with the study conducted among the U.S. Active Duty Service members in period 2000–2008 [17].

Peacekeeping operations did not significantly increase a risk for CTI among members of the SAF. Similar results were published in the study conducted among the U.S. military personnel deployed to Iraq and Afghanistan. The rates of CTI in this population were the same or lower than age- and year-matched U.S. rates reported by the CDC [23].

The aim of our research was to determine the CTI prevalence and RF in male and female military personnel of different ages, ranks, services/branches and years of active ser-

vice. Also, our study had a wide geographic sampling (eight barracks across Serbia). Because of that, the results of our study could be used as a recommendation for preventive measures and screening for the whole SAF.

Our study had two limitations. First, we did not collect samples for diagnosing extragenital CTIs. Another limitation was that we choose urogenital and endocervical swabs as samples for urogenital CTI detection (high sensitivity and high specificity). It is possible that some of our volunteers actually had some symptoms and because of that volunteered for painful swab collection. First limitation could lead to underestimation and other limitation could lead to overestimation of the CTI prevalence among the military population in the SAF. Consequently, more research on CTI in the the SAF military population is required.

Conclusion

CTI is significant problem in male and female military personnel in the SAF. The number of different sexual partners during the previous year was independently associated with CTI. These finding indicates that screening for CTI should be undertaken in the SAF. A screening program should be developed for all military personnel at the entry to the SAF as well as for the periodic rescreening. Such screening program has the potential to reduce rates of CTI in the SAF and to prevent morbidity due to this infection. In addition, the military should increase the prevention programming and knowledge about SII`s that encourages STI screening.

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Correlation between coagulation and inflammation state in patients with diabetes mellitus type 2 in relation to gender differences: is there any impact of eight-week exercise training?

Korelacija između koagulacionog i inflamatornog statusa kod bolesnika sa dijabetesom melitusom tip 2 u odnosu na polne razlike: da li postoji uticaj 8-nedeljnog vežbanja?

Divna Trebinjac*, Ivana Petronić[†‡], Nebojša Lalić[‡§], Dejan Nikolić[†‡]

University Hospital Ullevål, *Physical Medicine and Rehabilitation Department, Oslo, Norway; University Children's Hospital, [†]Physical Medicine and Rehabilitation Department, Belgrade, Serbia; University of Belgrade, [‡]Faculty of Medicine, Belgrade, Serbia; [§]Clinical Center of Serbia, Belgrade, Serbia

Abstract

Background/Aim. The hypercoagulable state and inflammation state in diabetics has been widely studied by previous researchers, but there is a lack of research about a possible impact of exercise training on this relationship. The aim of this study was to assess and compare correlation between the coagulation and inflammation status in patients with type 2 diabetes mellitus taking into account the gender differences as well as an impact of the 8-week exercise training on the correlation coefficient and parameters of the inflammation and coagulation state. **Methods.** A total of 60 patients in stable clinical condition and well-regulated diabetic status passed through all phases of the study. The exercise training included the exercise program as interval training with estimated intensity uphill to 75% of a maximal heart rate in particular individual, 5 times a week for 8 weeks, and walking for 30 minutes with a speed of 5 km/h, 5 times a week for 8 weeks. Further fibrinolytic, coagulation and inflammatory parameters were analyzed before and after the study: D-dimer, von Willebrand factor (vWF), fibrinogen, high sensitivity CRP (hs-CRP), leukocytes, thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT) and coagulation factors: FII, FV, FVII and FX. **Results.** Our research showed a statistically significant reduction in the mean vWF levels after intervention both at the males ($p < 0.001$) and females ($p < 0.001$). According to a correlation analysis between hs-CRP and fibrinogen, there was a positive correlation as baseline both at the males ($p < 0.05$, $r = 0.492$) and females ($p < 0.01$, $r = 0.516$) which became weaker in the males ($p < 0.01$, $r = 0.449$) and disappeared in the females ($p < 0.05$, $r = 0.059$) after intervention. The correlation which existed as baseline in the males between D-dimer and either hs-CRP ($p < 0.01$, $r = 0.633$) or fibrinogen ($p < 0.01$, $r = 0.673$) as well as the correlation between hs-CRP and FII ($p < 0.01$, $r = 0.728$), FV ($p < 0.05$, $r = 0.366$), FVII ($p < 0.05$, $r = 0.373$) coagulation as well as between D-dimer and FII ($p < 0.01$, $r = 0.851$), FVII ($p < 0.05$, $r = 0.367$)was absent in the females. Our research demonstrated a weakening correlations in the males after intervention between D-dimer and hs-CRP ($p < 0.05$, $r = 0.378$), between hs-CRP and FII ($p < 0.01$, $r = 0.501$), FV ($p < 0.05$, $r = 0.298$), FVII ($p < 0.05$, $r = 0.351$) as well as between D-dimer and FII ($p < 0.01$, $r = 0.759$), and FVII ($p < 0.05$, $r = 0.296$). The increase of the FX values ($p < 0.05$) in the females after intervention suggested the possible antiinflammatory effect of exercise training. **Conclusion.** According to previous research, the higher levels of vWF was associated with a risk of cardiovascular disease in people with type 2 diabetes mellitus and vWF may be a risk factor unique to these populations. We demonstrated that the 8-week exercise training can significantly reduce the value of vWF in the males and females, suggesting a potential beneficial effect on the endothelial function parameters. Our research demonstrated a stronger correlation between the coagulation and inflammation parameters as baseline in the males than in the females with type 2 diabetes mellitus. According to our results, the 8-week exercise training lead to a weakening of the strength of correlation between the coagulation and inflammation parameters in the males and complete disappearance of this correlation in the females, suggesting a unique effect of exercise training that should be explored in future research.

Key words:
diabetes mellitus, type 2; blood coagulation; inflammation; exercise; sex.

Correspondence to: Divna Trebinjac, University Hospital Ullevål, Physical Medicine and Rehabilitation Department, Hegdehaugsveien 36 C, 0352, Oslo, Norway. Email: divnatrebinjac@gmail.com

Apstrakt

Uvod/Cilj. Hiperkoagulacioni i inflamatorni status kod dijabetičara je opsežno proučavan u dosadašnjim istraživanjima, ali je mogući uticaj fizičkog treninga na međusobnu povezanost između koagulacionog i inflamatornog statusa nedovoljno istražen. Cilj našeg istraživanja bio je ispitivanje korelacija između inflamatornog i koagulacionog statusa kod bolesnika sa dijabetesom melitusom tip 2 u odnosu na pol, kao i uticaj fizičkog treninga na jačinu ovih korelacija i parametre koagulacionog i inflamatornog statusa. **Metode.** U istraživanje je bilo uključeno 60 bolesnika u stabilnom kliničkom stanju sa dobro regulisanim dijabetesom melitusom tip 2. Trening je primenjen u vidu intervalnog treninga sa maksimalnim intenzitetom od 75% maksimalne srčane frekvence, pet puta nedeljno u toku osam nedelja i treninga u vidu hoda brzinom od 5 km/h u trajanju od trideset minuta, pet puta nedeljno u toku osam nedelja. Sledeći fibinolitički, koagulacioni i inflamatorni parametri su analizirani pre i posle sprovedenog programa treninga: D-dimer, von Willebrand factor (vWF), fibrinogen, visokosenzitivni C-reaktivni protein (hs-CRP), leukociti, trombinsko vreme (TT), protrombinsko vreme (PT), aktivirano parcijalno tromboplastinsko vreme (APTT) i faktori koagulacije – FII, FV, FVII i FX. **Rezultati.** Naše istraživanje je pokazalo statistički značajno smanjenje nivoa vWF posle studije kod ispitanika muškog ($p < 0,001$) i ženskog pola ($p < 0,001$). Pozitivna korelacija između hs-CRP i fibrinogena, pokazana bazalno kod ispitanika muškog ($p < 0,05$, $r = 0,492$) i ženskog pola ($p < 0,01$, $r = 0,516$) koja bila je slabija kod ispitanika muškog pola ($p < 0,01$, $r = 0,449$), odnosno, gubila se kod ispitanica ženskog pola ($p < 0,05$, $r = 0,059$) nakon sprovedenog treninga. Pokazane su pozitivne bazalne korelacije kod ispitanika muškog pola između D-dimera sa jedne strane i hs-CRP ($p < 0,01$, $r = 0,633$) i fibrinogena ($p < 0,01$, $r = 0,673$) sa druge strane, kao i između hs-CRP i FII ($p < 0,01$, $r = 0,728$), FV ($p < 0,05$, $r = 0,366$), FVII ($p < 0,05$, $r = 0,373$, kao i između D-dimera i FII ($p < 0,01$, $r = 0,851$) i FVII ($p < 0,05$, $r = 0,367$), dok te korelacije nisu pokazane kod ispitanica ženskog pola. Naše istraživanje je kod ispitanika muškog pola pokazalo slabljenje jačine korelacija nakon sprovedenog treninga između D-dimera i hs-CRP ($p < 0,05$, $r = 0,378$), hs-CRP i FII ($p < 0,01$, $r = 0,501$), FV ($p < 0,05$, $r = 0,298$), FVII ($p < 0,05$, $r = 0,351$), kao i između D-dimera i FII ($p < 0,01$, $r = 0,759$), i FVII ($p < 0,05$, $r = 0,296$). Smanjenje nivoa FX kod ispitanica ženskog pola ($p < 0,05$) nakon sprovedenog treninga ukazuje na mogući antiinflamatorni efekat fizičkog treninga. **Zaključak.** Prema predhodnim istraživanjima, vWF može biti faktor rizika u populaciji dijabetičara i njegov povišeni nivo je povezan sa rizikom od nastanka kardiovaskularnog oboljenja. Mi smo pokazali da fizički trening u trajanju od osam nedelja može znacajno smanjiti nivo vWF kod oba pola, ukazujući na potencijalni povoljni efekat na parametre endotelne funkcije. Naše istraživanje je pokazalo veću jačinu korelacija između koagulacionih i inflamatornih parametara kod ispitanika muškog pola u odnosu na ispitanice ženskog pola. Prema našim rezultatima, osmonedeljni fizički trening dovodi do slabljenja jačine korelacija između koagulacionih i inflamatornih parametara kod ispitanika muškog pola i slabljenje ovih korelacija do potpunog gubitka kod ispitanica ženskog pola, ukazujući na jedinstveni efekat fizičkog treninga koji bi trebalo da bude ispitan u budućim istraživanjima.

Ključne reči:
dijabetes melitus, insulin-nezavisni; krv, koagulacija; zapaljenje; vežbanje; pol.

Introduction

It is estimated that by 2025 the number of diabetics worldwide will have affected 324 million people and will have an epidemic character [1]. Diabetes mellitus alters blood coagulation and platelet function which supports the suggestion that diabetes mellitus is a hypercoagulable state with changes in fibrinolysis, decreased fibrinolytic activity and increased thrombotic risk [2, 3]. Correlation between the coagulation factors in diabetics is more evident than in health subjects and this may be the reason for the more hypercoagulable conditions stated in diabetics. Dayer et al. [4] (2014) found that a path model or diagram for the coagulation factors were more complicated in diabetic patients than in normal individuals and conveyed that a sudden increase in the synthesis of each coagulation factors or their activation may trigger the initiation of coagulation cascade, leading to vascular clot formation with myocardial consequences.

Adipose tissue releases mediators that induce a chronic inflammation state and alterations in coagulation [5]. The metabolic syndrome is frequently accompanied by a prothrombotic state. This includes the elevated plasma levels of von Willebrand factor (vWF), and coagulation factors: FVIII, FVII and fibrinogen [6]. D-dimer and fibrinogen are known to be the thrombosis risk factors [7, 8]. Elevation of D-dimer may increase a risk of future myocardial infarction, stroke, and peripheral vascular disease [9]. D-dimer indicate a low grade of the prothrombotic risk in patients with diabetes mellitus type 2, but a higher risk of vascular complications [10]. In type 2 diabetic patients with or without vascular complications fibrinogen concentrations do not indicate remarkable difference and may not be an important causal factor for vascular complications [11], and it was found that diabetics had more fibrinogen in blood than healthy subjects [12]. vWF is an acute phase protein and its plasma level increases in systemic inflammation. Hemostatic imbalance may contribute to the development of cardiovascular disease in patients with type 2 diabetes mellitus. In patients with diabetes mellitus there is a state of hypofibrinolysis and increased levels of vWF [13]. The elevated levels of vWF are correlated with atherosclerosis and are associated with endothelial dysfunction in type 2 diabetes, as well as the development of diabetes in post-infarction patients [14, 15]. A recent research has shown that the increased vWF and D-dimer levels were associated with renal dysfunction in patients with type 1 diabetes, suggesting that endothelial dysfunction and hypercoagulability were associated with nephropathy in type 1 diabetes [16]. Regular exercise training has anti-inflammatory effects and can reduce the risk

of future thrombotic events [17]. The coagulation cascade plays a critical role in the development of cardiovascular disease. Exercise training is known to reduce cardiovascular disease risk and through improved coagulation profile may contribute to this reduction [18]. Single physical training has thrombotic effect that results in an increase of the number and activity of platelets, but regular exercise training attenuates these effects and acting suppression of coagulation [19].

The aim of this study was to assess and compare correlation between the coagulation and fibrinolytic state relating to gender differences in diabetics with type 2 diabetes mellitus as well as an impact of exercise training both on the coagulation and fibrinolytic state and correlation between them.

Methods

Study design and study protocol

The study examined the impact of eight-week exercise training (EWET) interval on markers of fibrinolysis, coagulation and inflammation in the patients with well-regulated diabetes mellitus type 2 with regard to gender differences. The study was designed as longitudinal observational study. The study was conducted in accordance with the Declaration of Helsinki. All the procedures were previously approved by the Institutional Review Board and Ethics Committee of the Faculty of Medicine, University of Belgrade (06-17512/62-12, No. 22/XII-4-1 dd 22.03.2012). Sixty patients, 35 males and 25 females were enrolled in the study according to the inclusions criteria. The purpose of the study was clearly explained to the patients. All the subjects were informed about the study protocol and they provided their written consent before the beginning of the study. The investigation was performed in accordance with the ethical standards and according to the national and international guidelines. For the purpose of the fibrinolytic and coagulation states assessment, we analyzed D-dimer, vWF, fibrinogen level, thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT) and coagulation factors: FII, FV, FVII and FX. The inflammation status was assessed by the high sensitivity C-reactive protein (hs-CRP) levels and leukocytes count. Both fibrinolytic and coagulation states were evaluated before and after EWET.

Physical exercise protocols

The training under supervision consisted of the aerobic training with 30 minutes of brisk walking with a speed of 5 km/h and the exercise training program. The exercise training was of an interval mode with intensity that was estimated according to 75% of a maximum heart rate. The rest time between exercise sessions was equivalent of the time spent on exercises. The patients walked and trained 5 times a week for 8 weeks. Each exercise session was completed in 45 minutes and consisted of warm up for 10 minutes at 50% of a maximum heart rate in particular individual, intervals of 25 minutes at 75% of a maximum heart rate in particular individual and 10 minutes cool-down period at 50% of a maximum heart rate in particular individual. The exercise program included flexibility exercise, balance exercise, stretching, circles going forward, circles going backward, hip flexors stretch, hip circles, arm circles, walking on toes and heals, lunge and trunk rotation exercise.

The patients were selected on the basis of their medical history and 60 patients with well-regulated diabetes passed through all phases of the study. The inclusions criteria were the age between 40 and 60 years, a stable clinical condition and well-regulated diabetic status as revealed by glycated haemoglobin (HbA1c) in the range of 6.0–8.0%.

The patients on warfarin or heparin, which might affect APTT and fibrinogen, were excluded from the study. The exclusion criteria were recent surgery or illness, cardiac arrhythmias, abnormal ECG during exercise treadmill testing before the study, diabetic cardiomyopathy, uncontrolled hypertension, uncompensated heart failure, severe valvular heart disease and musculoskeletal conditions that would hinder safe completion of the proposed exercise protocols.

Coagulation tests

The analyzed parameters were evaluated by the samples of drown blood in the patients before meal in the morning period before and after intervention (the first day of the beginning of the study and one day after the last training session). All samples were assayed in duplicate. Venous blood (4.5 mL) for the test of fibrinogen, D-dimer, FII, FV, FVII and FX, APTT, TT and PT was collected in a fasting state into cooled tubes (Vacutainer® system) using 3.2% trisodium citrate as an anticoagulant, after centrifugation at 2,500 g for 15 minutes. All coagulation tests were performed by using the Beckman Coulter ACL Elite Pro, Coagulation Analyzer. APTT and TT were expressed directly in seconds (s), within a normal range of 24.3–35.0 s and 11.0–17.8 s. The PT results were reported in seconds (11.8–15.1s). The coagulation factors were expressed as ratio in %: FII (50.0%–150.0%), FV (62.0%–139.0%), FVII (50.0%–129.0%), FX (77.0%–131.0%). The reference value for D-dimer was 255 ng/mL and for fibrinogen, it was from 2.0 to 4.8 g/L. The determination of vWF Ag was performed by using the automated hemostasis analyzer Siemens BCS-XP (Siemens Healthcare Diagnostics Inc. Marburg/Germany, von Willebrand reagent REF OUBD37. The vWF Ag result was reported in percentage of normality. The reference value for vWF was from 55% to 200%. All plasma samples were stored in the polypropylene tubes at -80°C until used for the measurement. The Le count with the reference value 3.4-9.7 x 10^9/L was determined by using the ADVIA 120 Hematology System, Siemens. The high sensitivity CRP values with the reference value 0–5 mg/L were analyzed by the commercial kits of enzyme immunosorbent assay (ELISA).

Statistical analysis

The evaluated parameters were presented as mean values with standard deviation (SD). The Student's *t*-test was used to assess a statistical difference between the mean values where a

distribution was shown to be normal and the statistical significance was set at $p < 0.05$. The Pearson's correlation coefficient test was done in order to establish correlation between the continuous variables. The Student's *t*-test was used for the paired and unpaired samples to assess a statistical difference between the mean values where distribution was shown to be normal. The significance level of 0.05 was used for the correlation tests. The statistical analyses were performed by using the Statistical Package for the Social Sciences (SPSS, USA), version 19.0.

Results

Analysis of the average values of the evaluated parameters before and after EWET in relation to the gender differences (Tables 1 and 2), revealed that there was a significant decrease in the values of vWF in the males (t = 3.488, df = 34, $p < 0.005$) and the females ($t = 3.601$, df = 24, $p < 0.001$) as well as the values of the TT in the males ($t = 4.303$, df = 34, $p < 0.001$) and the females ($t = 3.401$, df = 24, $p < 0.005$) after EWET. There was the significant increase of the values of FVII both in the males within the normal range ($t = -4.354$, df = 34, $p < 0.001$) and the females (t = -4.398, df = 24 p < 0.001), and the FX in the females within the normal range ($t = -2.685$, df = 24, $p < 0.05$) after EWET. Other parameters showed the non-significant changes during the evaluation period (fibrinogen, PT, D-dimer, FII, FV, Le, APTT values) ($p > 0.05$).

The average reduction in the vWF value in the males was 10.32 with 95% confidence interval (CI) of 3.488. The eta-squared value (0.26) showed a large effect of intervention.

The average reduction in the TT value in the males was 0.828 s with 95% CI of 4.303. The eta-squared value (0.35) showed a large effect of intervention.

The average increase in the value of factor FVII coagulation in the males was -11.45 with 95% of the confidence interval -4.364. The eta-squared value(0.35) showed a large effect of intervention.

The average reduction in the vWF value in the females was 15.88% with 95% confidence interval of 3.601%. The eta-squared value (0.35) showed a large effect of intervention.

The average reduction in the value of TT in the females was 0.92 s with confidence interval of 3.401s. The eta-squared value (0.32) showed a large effect of intervention.

The average increase in the value of FVII in the females was 17.924% with 95% CI of -4.398%. The eta-squared value (0.44) showed a large effect of intervention.

The average increase in the value of FX in the females was 6.74% with 95% CI of 2.685%. The eta-squared value (0.23) showed a large effect of intervention.

According to the analysis of the average baseline values of the evaluated parameters in relation to gender differences before EWET (Table 3), there was a significant difference in the mean values for vWF between the males (106.25 ± 32.61%) (t = -1.38, df = 19) and the females (124.28 ± 24.62%) ($t = -1.396$, df = 18.77) ($p < 0.05$) with the values within the normal range. Other parameters showed the non-significant changes as baseline (TT, fibrinogen, PT, D-dimer, F II, FV, FVII, FX, leukocytes, hs-CRP, APTT values) ($p > 0.05$) (Table 1).

According to the analysis of the average values for the evaluated parameters in relation to the gender differences after EWET (Table 4), there were the significant differences in mean values for fibrinogen between the males (4.75 ± 1.06 g/L) (t = -2.02, df = 58) and the females (5.27 ± 0.88 g/L) (t = -2.08, df = 56.57) ($p < 0.05$), FV between the males (113.88 ± 20.37%) (t = -2.12, df = 58) and the females (131.80 ± 22.63%) (t = -2.13, df = 52.24) ($p < 0.05$), FVII between the males (114.05 ± 19.01%) (t = -3.291, df = 58) and the females (131.80 ± 22.63%) (t = -3.196, df = 46.04) ($p < 0.005$), FX between the males (102.21 ± 16.58%) (t = -2.75, df = 58) ($p < 0.01$) and the females (113.08 ± 12.59%) (t = -2.88, df = 57.76) ($p < 0.01$) with all values within the normal range.

According to the analysis of correlation between baseline inflammation and coagulation parameters in the males before EWET (Table 5), there was a very strong positive correlation between D-dimer and FII (t = 0.85, $p < 0.01$). There was a strong positive correlation between hs-CRP and FII (t = 0.78, $p < 0.01$), and hs-CRP and D-dimer (t = 0.63, $p < 0.01$), D-dimer and fibrinogen (t = 0.67, $p < 0.01$), fibrinogen and FII (t = 0.64, $p < 0.01$). There is a week positive correlation between hs-CRP and FV (r = 0.366, $p < 0.05$), hs-CRP and FVII (r = 0.373, $p < 0.05$), hs-CRP and Le (r = 0.355, $p < 0.05$), and FVII and D-dimer (r = 0.37, $p < 0.05$). There were the significant positive moderate correlations between hs-CRP and fibrinogen (r = 0.49, $p < 0.05$), hs-CRP and FX (r = 0.40, $p < 0.05$), fibrinogen and FV coagulation (r = 0.47, $p < 0.01$).

According to the analysis of correlation between baseline inflammation and coagulation parameters in the females before EWET (Table 6), there were the significant moderate positive correlations between hs-CRP and fibrinogen (r = 0.52, $p < 0.05$), and FII (r = 0.52, $p < 0.01$).

According to the analysis of correlation between the inflammation and coagulation parameters in the males after EWET (Table 7), there were strong positive correlations between D-dimer and fibrinogen (r = 0.72, $p < 0.01$), D-dimer and FII (r = 0.76, $p < 0.01$), fibrinogen and FII (r = 0.61, $p < 0.05$). Also, there were the significant moderate positive correlations between CRP and fibrinogen (r = 0.45, $p < 0.01$), Le and fibrinogen (r = 0.52, $p < 0.01$), hs-CRP and FII (r = 0.50, $p < 0.05$). There were the significant weak positive correlations between CRP and FVII (r = 0.35, $p < 0.05$), fibrinogen and FV (r = 0.35, $p < 0.05$), D-dimer and FV (r = 0.39, $p < 0.05$).

According to the analysis of correlation between the inflammation and coagulation parameters in the females after EWET (Table 8), the negative correlation between D-dimer and hs-CRP (r = -0.46, $p < 0.05$) was demonstrated.

Table 1

Average values of evaluated parameters before and after the 8-week exercise training (EWET) in the males with diabetes mellitus type 2

Parameters relative to EWET	Mean ± SD	*p*
vWF (%) (55–200) #		
before	106.26 ± 32.618	< 0.001*
after	95.94 ± 32.685	
Thrombin time (s) (11.0–17.8)#		
before	14.63 ± 1.34	< 0.001*
after	13.84 ± 1.07	
Fibrinogen (g/L) (2.0–4.8) #		
before	4.65 ± 1.09	> 0.05
after	4.75 ± 1.06	
Prothrombin time (s) (11.8–15.1) #		
before	13.86 ± 0.99	> 0.05
after	13.69 ± 0.95	
D-dimer (ng/mL) (255)#		
before	206.52 ± 141.47	> 0.05
after	206.58 ± 137.85	
Factor II (%) (50–150) #		
before	130.20 ± 136.32	> 0.05
after	134.02 ± 119.07	
Factor V (%) (62–139) #		
before	112.76 ± 19.03	> 0.05
after	113.88 ± 20.37	
Factor VII (%) (50–129)		
before	102.60 ± 22.84	< 0.001*
after	114.05 ± 19.01	
Factor X (%) (77–131)#		
before	97.71 ± 19.70	> 0.05
after	102.21 ± 16.58	
Hs-CRP (mg/L) (0–5)#		
before	1.80 ± 2.55	> 0.05
after	2.20 ± 3.25	
APTT (s) (24.3–35.0)#		
before	26.50 ± 2.19	> 0.05
after	26.34 ± 2.35	
Leukocytes (10^9/L) (3.4–9.7)#		
before	6.85 ± 2.14	> 0.05
after	7.13 ± 1.79	

#normal value; *significant correlation at $p < 0.05$; vWF – won Willebrand factor; CRP – C-reactive protein; Hs-CRP – high sensitivity CRP; APTT – activated partial thromboplastin time; SD – standard devations.

Table 2

Average values of the evaluated parameters before and after the 8-week exercise training (EWET) in the females with diabetes mellitus type 2

Parameters relative to EWET	Mean ± SD	*p*
vWF (%) (55–200) #		
before	124.28 ± 24.62	< 0.001*
after	108.40 ± 23.65	
Thrombin time (s) (11.0–17.8) #		
before	14.94 ± 1.32	< 0.005*
after	14.02 ± 1.05	
Fibrinogen (g/L) (2.0–4.8) #		
before	5.21 ± 1.20	> 0.05
after	5.27 ± 0.88	
Prothrombin time (s) (11.8–15.1) #		
before	13.56 ± 0.72	> 0.05
after	13.64 ± 0.74	
D-dimer (ng/L) (255) #		
before	208.16 ± 55.30	> 0.05
after	211.64 ± 94.86	
Factor II (%) (50–150) #		
before	118.71 ± 29.88	> 0.05
after	121.80 ± 15.79	
Factor V (%) (62–139) #		
before	118.69 ± 21.95	> 0.05
after	125.15 ± 20.12	
Factor VII (%) (50–129) #		
before	113.87 ± 23.10	< 0.001*
after	131.80 ± 22.63	
Factor X (%) (77–131) #		
before	106.34 ± 12.74	< 0.05*
after	113.08 ± 12.59	
Hs-CRP(mg/L) (0–5) #		
before	2.55 ± 2.57	> 0.05
after	2.49 ± 2.04	
APTT (s) (24.3–35.0) #		
before	25.62 ± 2.55	> 0.05
after	25.48 ± 2.49	
Leukocytes(10^9/L) (3.4–9.7) #		
before	7.44 ± 1.60	> 0.05
after	6.91 ± 1.45	

#normal value; *significant correlation at $p < 0.05$; vWF – won Willebrand factor; CRP – C-reactive protein; Hs-CRP – high sensitivity CRP; APTT – activated partial thromboplastin time; SD – standard devations.

Table 3

Average values of the evaluated parameters before the 8-week exercise training (EWET) in the males and females with diabetes mellitus type 2

Parameters	Sex	Mean ± SD	*p*
vWF (%)	m	106.26 ± 32.61	< 0.05*
(55–200) #	f	124.28 ± 24.62	
Thrombin time (s)	m	14.63 ± 1.34	> 0.05
(11.0–17.8)	f	14.94 ± 1.32	
Fibrinogen (g/L)	m	4.65 ± 1.09	> 0.05
(2.0–4.8) #	f	5.21 ± 1.20	
Prothrombin time(s)	m	13.86 ± 0.99	> 0.05
(11.8–15.1) #	f	13.56 ± 0.72	
D-dimer (ng/L)	m	206.52 ± 141.47	> 0.05
(255)	f	208.16 ± 55.30	
Factor II (%)	m	130.20 ± 136.32	> 0.05
(50–150) #	f	118.71 ± 29.88	
Factor V (%)	m	112.76 ± 19.03	> 0.05
(62–139) #	f	118.69 ± 21.95	
Factor VII (%)	m	102.60 ± 22.84	> 0.05
(50–129) #	f	113.87 ± 23.10	
Factor X (%)	m	97.71 ± 19.70	> 0.05
(77–131) #	f	106.34 ± 12.74	
Hs-CRP (mg/L)	m	1.80 ± 2.55	> 0.05
(0–5) #	f	2.55 ± 2.57	
APTT (s)	m	26.50 ± 2.19	> 0.05
(24.3–35.0) #	f	25.63 ± 2.55	
Leukocytes (10^9/L)	m	6.85 ± 2.14	> 0.05
(3.4–9.7) #	f	7.44 ± 1.60	

***Significant correlation at $p < 0.05$; m – males; f – females.**
For other abbreviations see under Tables 1 and 2.

Table 4

Average values of the evaluated parameters after the 8-week exercise training (EWET) in the males and females with diabetes mellitus type 2

Parameters	Sex	Mean ± SD	*p*
vWF (%)	m	95.94 ± 32.685	> 0.05
(55–200) #	f	108.40 ± 23.656	
Thrombin time (s)	m	13.84 ± 1.07	> 0.05
(11.0–17.8)	f	14.02 ± 1.05	
Fibrinogen (g/L)	m	4.75 ± 1.06	< 0.05*
(2.0–4.8) #	f	5.27 ± 0.88	
Prothrombin time(s)	m	13.69 ± 0.95	> 0.05
(11.8–15.1) #	f	13.64 ± 0.74	
D-dimer (ng/L)	m	211.42 ± 138.79	> 0.05
(255)	f	211.64 ± 94.86	
Factor II (%)	m	134.02 ± 119.07	> 0.05
(50–150) #	f	121.80 ± 15.79	
Factor V (%)	m	113.88 ± 20.37	< 0.05*
(62–139) #	f	125.15 ± 20.12	
Factor VII (%)	m	114.05 ± 19.01	< 0.005*
(50–129) #	f	131.80 ± 22.63	
Factor X (%)	m	102.21 ± 16.58	< 0.01*
(77–131) #	f	113.08 ± 12.59	
Hs-CRP (mg/L)	m	2.20 ± 3.25	> 0.05
(0–5) #	f	2.55 ± 1.97	
APTT (s)	m	26.34 ± 2.35	> 0.05
(24.3–35.0) #	f	25.48 ± 2.49	
Leukocytes (10^9/L)	m	7.13 ± 1.79	> 0.05
(3.4–9.7) #	f	6.91 ± 1.45	

***Significant correlation at $p < 0.05$.**
For other abbreviations see under Tables 1 and 2.

Table 5

Correlation between the baseline inflammation and coagulation parameters before the 8-week exercise training (EWET) in the males with diabetes mellitus type 2

Parameters before	CRP		D-dimer		Fibrinogen		vWF	
EWET	test values	*p*	test values	*p*	test values	*p*	test values	*p*
Fibrinogen	0.492*	< 0.05	0.673**	< 0.01	1		-0.044	> 0.05
Factor II	0.728**	< 0.01	0.851**	< 0.01	0.637**	< 0.01	-0.211	> 0.05
Factor V	0.366**	< 0.05	0.327	> 0.05	0.467**	< 0.01	-0.185	> 0.05
Factor VII	0.373*	< 0.05	0.367*	< 0.05	0.174	> 0.05	-0.021	> 0.05
Factor X	0.400*	< 0.05	0.232	> 0.05	0.152	> 0.05	0.093	> 0.05
vWF	-0.144	> 0.05	-0.220	> 0.05	-0.044	> 0.05	1	
APTT	0.118	> 0.05	0.043	> 0.05	0.182	> 0.05	-0.007	> 0.05
Thrombin time	-0.070	> 0.05	-0.130	> 0.05	-0.542**	< 0.01	0.022	> 0.05
Prothrombin time	-165	> 0.05	-0.242	> 0.05	-0.385	> 0.05	-0.012	> 0.05
Leukocytes	0.355*	< 0.05	0.203	> 0.05	0.298	> 0.05	-0.292	> 0.05
Hs-CRP	1		0.633**	< 0.01	0.492*	> 0.05	-0.144	> 0.05
D-dimer	0.633**	< 0.01	1		0.673**	< 0.01	-0.220	> 0.05

***Correlation is significant at the 0.05 level (2-tailed);**Correlation is significant at the 0.01 level (2-tailed).**
For other abbreviations see under Table 1.

Table 6

Correlation between the inflammation and coagulation parameters before the 8-week exercise training (EWET) in the females with diabetes mellitus type 2

Parameters before EWET	CRP		D-dimer		Fibrinogen		vWF	
	test values	*p*	test values	*p*	test values	*p*	test values	*p*
Fibrinogen	0.516*	< 0.01	-0.172	> 0.05	1		-0.224	> 0.05
Factor II	0.069	> 0.05	-0.294	> 0.05	0.518**	< 0.01	-0.206	> 0.05
Factor V	0.025	> 0.05	-0.083	> 0.05	0.158	> 0.05	0.370*	< 0.05
Factor VII	0.021	> 0.05	0.140	> 0.05	0.174	> 0.05	0.383*	< 0.05
Factor X	0.050	> 0.05	-0.092	> 0.05	0.052	> 0.05	0.384*	< 0.05
vWF	0.100	> 0.05	0.127	> 0.05	-0.224	> 0.05	1	
APTT	-0.213	> 0.05	-0.238	> 0.05	-0.214	> 0.05	-0.139	> 0.05
Thrombin time	-0.435*	< 0.05	-0.065	> 0.05	-0.752**	< 0.01	0.107	> 0.05
Prothrombin time	-0.318	> 0.05	-0.141	> 0.05	-0.074	> 0.05	-0.297	> 0.05
Leukocytes	-0.045	> 0.05	0.128	> 0.05	0.308*	< 0.05	-0.286	> 0.05
Hs-CRP	1		-0.372	> 0.05	0.516*	< 0.05	0.100	> 0.05
D-dimer	-0.372	> 0.05	1		-0.172	> 0.05	0.127	0.05

***Correlation is significant at the 0.05 level (2-tailed);**Correlation is significant at the 0.01 level (2-tailed).**
For other abbreviations see under Tables 1 and 2.

Table 7

Correlation between the inflammation and coagulation parameters after the 8-week exercise training (EWET) in the males with diabetes mellitus type 2

Parameters after EWET	CRP		D-dimer		Fibrinogen		vWF	
	test values	*p*	test values	*p*	test values	*p*	test values	*p*
Fibrinogen	0.449**	< 0.01	0.722**	< 0.01	1		-0.039	> 0.05
Factor II	0.501**	< 0.01	0.759**	< 0.01	0.607**	< 0.01	-0.114	> 0.05
Factor V	0.297	> 0.05	0.389*	< 0.05	0.348*	< 0.05	-0.052	> 0.05
Factor VII	0.351*	< 0.05	0.296	> 0.05	0.232	> 0.05	-0.144	> 0.05
Factor X	0.267	> 0.05	0.430**	< 0.01	0.336*	< 0.05	-0.170	> 0.05
vWF	-0.101	> 0.05	0.055	> 0.05	-0.039	> 0.05	1	
APTT	0.220	> 0.05	-0.120	> 0.05	0.198	> 0.05	-146	> 0.05
Thrombin time	-0.083	> 0.05	-0.034	> 0.05	-0.340	> 0.05	-0.033	> 0.05
Prothrombin time	-0.195	> 0.05	-0.174	> 0.05	-0.262	> 0.05	-0.296	> 0.05
Leukocytes	0.252	> 0.05	0.363*	< 0.05	0.523**	< 0.01	0.236	> 0.05
Hs-CRP	1		0.378*	< 0.05	0.449*	< 0.05	-0.101	> 0.05
D-dimer	0.378*	< 0.05	1		0.722**	< 0.01	-0.041	> 0.05

***Correlation is significant at the 0.05 level (2-tailed);**Correlation is significant at the 0.01 level (2-tailed).**
For other abbreviations see under Tables 1 and 2.

Table 8

Correlation between the inflammation and coagulation parameters after the 8-week exercise training (EWET) in the females with diabetes mellitus type 2

Parameters after EWET	CRP		D-dimer		Fibrinogen		vWF	
	test values	*p*	test values	*p*	test values	*p*	test values	*p*
Fibrinogen	0.059	> 0.05	0.006	> 0.05	1		-0.115	> 0.05
Factor II	-0.068	> 0.05	-0.413*	< 0.05	0.432*	< 0.05	-0.171	> 0.05
Factor V	-0.123	> 0.05	-0.224	> 0.05	0.031	> 0.05	-0.042	> 0.05
Factor VII	0.011	> 0.05	-0.116	> 0.05	-0.136	> 0.05	0.105	> 0.05
Factor X	-0.053	> 0.05	-0.126	> 0.05	0.082	> 0.05	-0.138	> 0.05
vWF	0.059	> 0.05	-0.018	> 0.05	0.102	> 0.05	1	
APTT	-0.086	> 0.05	0.272	> 0.05	0.031	> 0.05	-0.173	> 0.05
Thrombin time	-0.010	> 0.05	0.104	> 0.05	-0.144	> 0.05	-0.108	> 0.05
Prothrombin time	-0.208	> 0.05	0.400	> 0.05	0.109	> 0.05	-0.308	> 0.05
Leukocytes	-0.327	> 0.05	0.058	> 0.05	0.180	> 0.05	0.013	> 0.05
Hs-CRP	1		-0.465*	< 0.05	0.059	> 0.05	0.059	> 0.05
D-dimer	-0.465*	< 0.05	1		0.006	> 0.05	-0.018	> 0.05

***Correlation is significant at the 0.05 level (2-tailed);**Correlation is significant at the 0.01 level (2-tailed).**
For other abbreviations see under Tables 1 and 2.

Discussion

The present study was undertaken to explore the correlation between the inflammation and coagulation state as well as an impact of exercise training on this correlation in diabetics. WF is associated with cardiovascular disease, type 2 diabetes mellitus and insulin resistance. The higher levels of vWF are associated with a risk of cardiovascular disease in people with type 2 diabetes mellitus or insulin resistance, which suggests that vWF may be a risk factor unique to these populations. In addition, the elevated levels of vWF are associated with an increased risk of cardiovascular disease in a community-based sample, even after accounting for traditional cardiovascular disease risk factors [20].

Our study showed a significant decrease in the values of vWF both in the males ($p < 0.005$) and females ($p < 0.001$) with diabetes mellitus type 2 after EWET and these results correlated with recent researches. Creighton et al. [21] explored the effects of an acute resistance exercise test on the primary hemostatic system both in resistance-trained and untrained individuals and concluded that reduced vWF in both groups may be attributed to the training status. Androulakis et al. [22] examined whether a high volume of training could lead to the endothelial activation and/or dysfunction in professional soccer players due to exercise-induced oxidative stress and vWF antigen plasma levels were measured 1 day before and after 7 weeks of strenuous exercise. It was showed that the mean vWF Ag plasma levels were significantly decreased from 95.1% ± 26% to 88.3% ± 27.2% at the end of the experimental period ($p = 0.018$), suggesting a potential beneficial effect on the endothelium. According to Jahangard et al. [23], vWF showed a significant reduction after 10 sessions of submaximal aerobic cycling in sedentary healthy postmenopausal women. We found the the significant differences in the mean values for vWF between the males (106.25 ± 32.61%) and the females (124.28 ± 24.62%) ($p < 0.05$) as baseline with a higher value in the females within the reference value.

We showed the significant decreases in the values of TT both in the males ($p < 0.001$) and females ($p < 0.005$) after EWET. Our findings demonstrated no differences in APTT, PT and FII coagulation in diabetics and they were consistent with the results obtained by Lamprechts et al. [24] that showed no statistically significant differences between pre- and post a single bout of walking exercise in APTT, FII coagulation and PT in the obese women. A short-term exercise in healthy subjects is usually associated with a significant shortening of APTT [25, 26], but according to our results there was no differences in APTT after regular eight- week training. According to Lockard et al. [18] plasma prothrombin fragment was found to decrease significantly with exercise training three times a week in six months.

We found that the values of fibrinogen were distinctly above normal in the females both as baseline and after study and similar findings were reported by Kafle and Shrestha [12]. They found a significantly higher fibrinogen in patients with diabetes than in controls and showed that fibrinogen was significantly higher in diabetic patients with coronary artery disease than in those patients who had only diabetes or coronary artery disease. We found no changes in fibrinogen concentration after intervention and these findings correlated with a research of Bizheh and Jaafari al. [27] who examined an effect of a single bout resistance exercise in sedentary middle aged men on fibrinogen. According to a recent investigation, physical training reduced fibrinogen concentration in patients with coronary heart diseases who had long-term physical training. It was determined that fibrinogen concentration significantly increased after physical load in all the treatment stages both in the coronary heart disease and the control group, while fibrinogen concentration gradually decreased in the group of the trained patients after 1 year [28], supporting that physical training lasting for a longer period could effectively reduce fibrinogen concentration.

A recent study showed a correlation between hs-CRP and D-dimer in patients with pulmonary embolism [29]. According to our research, a stronger baseline correlation was found between hs-CRP and D-dimer than between hs-CRP and fibrinogen in the males as baseline. In the females, a strong positive correlation between hs-CRP and fibrinogen as baseline was demonstrated, but there was no correlation between those parameters after the study. In the females, a negative correlation was demonstrated between hs-CRP and D-dimer both as baseline and after the study. According to the Speedwell Study, a positive correlation was shown between hs-CRP and D-dimer and a much stronger association between hs-CRP and fibrinogen in heart disease [30], and these findings correlate with our findings as baseline. Also, according to a recent research, hs-CRP correlated positively with fibrinogen and D-dimer in hemodialysis patients [31]. These findings correlate with our findings which showed a strong positive correlation between hs-CRP and either D-dimer or fibrinogen in the males as baseline. In addition, we demonstrated a weakness of this correlation in the males after EWET.

According to Long et al. [32] , D-dimer did not correlate positively with fibrinogen in diabetics. In our study, there was no correlation found between D-dimer and fibrinogen but only in the females. We demonstrated a strong correlation between D-dimer and fibrinogen in the males both before and after the study.

FII is the main cause for hypercoagulable state and the last target of coagulation cascade either from intrinsic or extrinsic origin and they play a determinant role in initiation of vascular complications in diabetics, while D-dimer indicate a higher risk of vascular complications in patients with diabetes mellitus type 2. According to our study, it was demonstrated that a strong correlation between D-dimer and FII in the males became moderate after the study. In the females, there was a negative correlation between D-dimer and FII either as baseline or after the study.

We demonstrated no change in hs-CRP concentration in our study which correlated with the research done by Levinger et al. [33] on 56 middle-aged men and women. They showed that 10 weeks of resistance training did not alter significantly the hs-CRP expression. In our research, we demonstrated a strong correlation between hs-CRP and FII in the males as baseline, and a moderate correlation after intervention, suggesting a potential benefit of EWET on the rela-

tionship between coagulation and inflammation. In addition, we showed no correlation between hs-CRP and FII in the females either before or after the study, pointing to a weaker association between coagulation and inflammation in the females in comparison with the males.

According to the analysis of correlation between baseline inflammation and coagulation parameters in the males before our study, there was a strong positive correlation between D-dimer and either FII or fibrinogen as baseline that stayed strong after the intervention, while those correlation were negative in the females both before and after the study. We found a weakness in correlation between hs-CRP and either fibrinogen, FII, FV, FVII, FX, Le or D-dimer in the males after the study, supporting effectiveness of the intervention.

In a recent research, Dayer et al. [34] obtained correlation between FV and vWF in diabetics which was absent in a normal mode, and, in addition, FV may bind to vWF [4], and both findings correlated with our findings. We demonstrated a moderate correlation between vWF and either FV, FII or FX but only in the females, which completely disappeared after the study, suggesting potential effect of the 8-week training. We obtained moderate correlation between fibrinogen and FV in the males as baseline, and a weak correlation after 8-week training suggesting both the higher coagulation state and anti-coagulation effects of intervention in the males, while there was no correlation between fibrinogen and FV in the females either before or after the training, suggesting a lower coagulation state in the females.

A recent study which investigated the correlation of the coagulation indicators with the inflammatory markers for sepsis in hematologic malignancy patients demonstrated that the level of procalcitonin positively correlated with the APTT and D-dimer level [35]. Our study showed the significant moderate positive correlation between hs-CRP and fibrinogen both in the males and females as baseline as well as in the males after EWET, but no correlation between hs-CRP and fibrinogen in the females after the 8-week training. Similar results were reported by Thor et al. [36] as a strong positive correlation between hs-CRP and fibrinogen particularly in diabetics which correlated with our findings. In addition, they reported a correlation between hs-CRP and either fibrinogen or vWF in diabetics. According to them, there was no correlation between fibrinogen and other markers of hypercoagulability, thrombin-anti thrombin, prothrombin and D-dimer, although the last three ones correlated with each other. In our study there was no correlation between vFW and hs-CRP. We showed a correlation between hs-CRP and fibrinogen in the males both as baseline and after the training, and, in addition, we showed a positive correlation between hs-CRP and fibrinogen as baseline in the females, which became not significant after the training. This result supports an anti-inflammatory effect of EWET.

According to Alehagen et al. [37], patients with suspected heart failure and low plasma concentrations of FII, FVII and FXI had significantly higher mortality rate during the follow-up period of 10 years as compared with those with a higher plasma concentrations. Increasing in plasma concentrations of FII and FVII after exercise training may indicate a protective effect of training on mortality and a protective effect of exercise training on hemorrhage. We demonstrated significant increase within the normal range of FVII in the males and little above normal range in the females after EWET, suggesting a protective effect of training on mortality. According to Ruiz-Saez [38] afibrinogenemia, FVII or FXI deficiencies are the ones most commonly associated with venous or arterial thrombosis. FVIIa is significantly higher in the patients with type 2 diabetes mellitus [39]. FVII deficiency is an uncommon coagulation disorder that patient usually presents with bleeding diathesis, but thrombotic event was reported in patients with acute ishemic stroke [40]. Increasing of the FVII levels in our study may indicate possible reduction of a risk for venous or arterial thrombosis after 8-week training.

The function of FXa is unclear. Interesting results were recently reported by Ku and Bae [41]. The expression level of the secretory group IIA phospholipase A2 is elevated in inflammatory diseases and lipopolysaccharide upregulates the expression. FXa suppressed the activation of cytosolic phospholipase A2 and extracellular signal-regulated kinase by lipopolysaccharide [41]. According to a study by Bukowska et al. [42], FXa mediates inflammatory signaling in atrial tissue by inducing an inflammatory signaling by activation of protease-activated receptors. Gleeson et al. [43] pointed out to the novel function concerning the FX as an endogenous, receptor-associated protein-sensitive, protease-activated receptor 2-dependent regulator of myeloid cell proinflammatory cytokine production. Further, exposure to the FX significantly impairs pro-inflammatory cytokine production. The FX inhibits the nuclear factor-kappa B activation in THP-1 reporter cells requires phosphatidylinositol 3-kinase activity for its anti-inflammatory effect [43]. We demonstrated a significant increase of the FX level in the females within the normal range after 8-week training. In addition, we demonstrated moderate positive correlation between hs-CRP and FX in the males as baseline that became weak correlation after the study, and none correlation between hs-CRP and FX in the females. The increase in the plasma concentration of the FX after EWET only in the females could suggest the possible anti-inflammatory effect of exercise training and should be examined in future research.

The main limitation of the present study is the relatively small number of patients, but the study enrolled both men and women in an adequate number to obtain the statistically significant results. The major advantage of the present study is an investigation of correlation between the inflammation and coagulation state in relation to two sexes with diabetes, which was insufficient in the previous researches. This longitudinal study may bring some new findings regarding effects of exercise training on treatment of patients with type 2 diabetes mellitus.

Conclusion

We demonstrated a statistically significant reduction in the mean vWF levels after EWET training both in the males and females with type 2 diabetes mellitus, suggesting a po-

tential beneficial effect on the endothelial function parameters. According to our research, a stronger correlation between the coagulation and inflammation parameters in the males than in the females as baseline was obtained, and, in addition, we obtained the weakening of correlation between the coagulation and inflammation parameters both in males and females after intervention, suggesting an anti-inflammatory and anticoagulant effect of 8-week training. The effect of exercise training on correlation between the coagulation and inflammation state in the patients with both well-controlled and poorly-controlled type 2 diabetes mellitus should be explored in future.

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Multidetector computed tomography (MDCT) estimation of prevalence and anatomic characteristics of the sternal body foramen in the population of central Serbia

Procena učestalosti i anatomskih karakteristika otvora tela sternuma u populaciji centralne Srbije pomoću multidetektorske kompjuterizovane tomografije (MDCT)

Maja Vulović*†, Ivana Živanović-Mačužić*†, Dejan Jeremić*†, Nela Djonović‡, Aleksandar Radunović§, Milan Jovanović†, Bojan Milošević‖, Zoran Aleksić¶, Ivana Stanković, Radiša Vojinović††**

University of Kragujevac, Faculty of Medical Sciences, *Department of Anatomy, ‡Department of Hygiene and Ecology, ‖Department of Surgery, ††Department of Radiology, Kragujevac, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; Military Medical Academy, §Clinic for Orthopedic Surgery, Belgrade, Serbia; University Clinical Center of Republic of Srpska,¶Clinic for General and Abdominal Surgery, Bosnia and Herzegovina; **Ambulance Brigade of Guards, Belgrade, Serbia

Abstract

Background/Aim. The most frequent form of sternal defects is a single foramen, usually located at the distal half of the sternal body, with prevalence that varies among different ethnic populations. Clinical importance of these defects arises from various diagnostic and therapeutic sternal treatments and close location of heart, lungs and other vital organs of the chest cavity. The aim of this study was to determine the prevalence and morphometric characteristics of the sternal midline foramen in the population of central Serbia. **Methods**. The multidetector computed tomography (MDCT) chest images of 422 patients of both genders were analyzed. The radiological imaging was performed on 64-slice MDCT scanner (Aquilion 64, Toshiba, Japan). All scans were performed in the axial plane, with subsequent multiplanar reconstruction (MPR). Due to the angulation of the sternal body coronal curved-planar, the images were obtained in order to show the whole length of the sternum and the vertical diameter of the sternal foramen**.** The measurements were done using the commercially available software (Imaging Software ver. 4.1.14.0, Vital-Images). **Results.** The solitary foramen, located in the distal segment of the sternal body, was detected in 24 patients, representing 5.9% of the observed population with slightly higher prevalence in males. The average size of foramen was 3.9 × 4.2 mm. The mean distance from the skin was 12.7 ± 3.3 mm, the distance from skin to pericardium was on average 37.3 ± 8.2 mm, while the average distance from skin to pleura was 25 ± 5.9 mm. The average depth of foramen 8.7 ± 2 mm, while the mean distance from the posterior surface of foramen to pericardium was 12.7 ± 9.1 mm. **Conclusion.** The results presented in this paper confirmed the prevalence of 5.9% regarding the midline sternal foramen in the observed population. Serious complications of the sternal puncture could be prevented by prior MDCT imaging.

Key words:
sternum; musculoskeletal abnormalities; multidetector computed tomography; prevalence; sternotomy; risk assessment; serbia.

Apstrakt

Uvod/Cilj. Jedna od najučestalijih anatomskih anomalija sternuma je otvor, najčešće jedan, obično lociran u donjem delu tela grudne kosti. Učestalost ove malformacije sternuma varira u zavisnosti od ispitivane etničke populacije. Klinički značaj otvora tela sternuma proizilazi iz činjenice da se obično nalazi u neposrednoj blizini srca, pluća i drugih vitalnih organa grudne duplje, kao i mogućnosti nastanka po život opasnih komplikacija, usled povrede ovih organa, prilikom dijagnostičkih i terapeutskih procedura na stenumu. Cilj ovog istraživanja bio je da se utvrdi učestalost i morfometrijske karak-

Correspondence to: Ivana Živanović-Mačužić, University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Anatomy, Svetozara Markovića 69, 34 000 Kragujevac, Serbia. E-mail: ivanaanatom@yahoo.com

teristike otvora tela grudne kosti u populaciji centralne Srbije. **Metode.** U studiji su analizirani snimci multi-detektorske kompjuterizovane tomografije (MDCT) grudnog koša kod 422 pacijenata oba pola. Pregledi su obavljeni na 64-slajsnom MDCT aparatu (Aquilion 64, Toshiba, Japan). Sva skeniranja su izvršena u aksijalnoj ravni, uz naknadne multiplanarne rekonstrukcije (MPR). S obzirom na angulaciju sternuma u predelu tela korišćene su kose koronalne ravni da bi se prikazala cela dužina sternuma, kao i visina sternalnog foramena. Merenje je izvršeno upotrebom komercijalno dostupnog softvera (Imaging Software ver. 4.1.14.0, Vital-Images). **Rezultati.** Otvor tela grudnog koša, lociran u distalnom segmentu tela sternuma uočen je kod 24 pacijenta, odnosno sa učestalošću od 5,9% u ispitivanoj populaciji, a sa nešto većom učestalošću kod muškaraca. Prosečne dimenzije otvora bile su 3,9 × 4,2 mm. Srednja vrednost udaljenosti kože do otvora iznosila je 12,7 ± 3,3 mm, udaljenosti kože do perikarda 37,3 ± 8,2 mm, dok je prosečno rastojanje od kože do pleure iznosilo 25 ± 5,9 mm. Prosečna dubina otvora iznosila je 8,7 ± 2 mm, dok je srednja vrednost rastojanja od zadnje ivice otvora do perikarda iznosila 12,7 ± 9,1 mm. **Zaključak**. Studijom je utvrđena učestalost otvora tela grudne kosti od 5,9% u ispitivanoj populaciji. MDCT pregledi grudnog koša, koji prethode intervencijama na grudnoj kosti, mogu biti od značaja za prevenciju komplikacija punkcije sternuma.

Ključne reči:
sternum; mišićno-skeletne anomalije; tomografija, kompjuterizovana, multidetektorska; prevalenca; rizik, procena; srbija.

Introduction

The human sternum is the flat bone of the anterior thoracic wall, adjacent to pleura, pericardium and the great vessels of thoracic cavity. Embriologically, sternum is composed of two mesenchymal bars that are merged in the median line to form a pre-cartilage sternal lamina, divided into three parts: manubrium, corpus sterni and xiphoid processus. Any deviation from this process may form various sternal defects, including clefts, fissures and sternal foramina [1, 2].

Prevalence of these sternal defects may differ in various countries and is generally believed to vary between 2% and 8% in the general population [3], although it is reported to be as high as 13.8% [4]. The most frequent form is a single sternal foramen, usually located at the distal half of the sternal body, at the level of the fourth or fifth intercostal space [2, 5].

The clinical importance of these defects arises from the sternal punctures, acupuncture treatment and close location of heart, lungs and other vital organs of the chest cavity [6]. The most serious complications are heart tamponade [7] and puncture of the ascending aorta [8].

The current study is undertaken in order to ascertain the frequency, morphometric characteristics of the sternal body foramen and its distance from the adjacent organs of thoracic cavity, analyzing the multi-detector computed tomography (MDCT) images in the population of central Serbia.

Methods

The study was designed as a retrospective descriptive non-randomized observational study, that used data from MDCT patients' chest images archived in the hospital information system for data archiving (PACS). The patients were examined at the Department of Diagnostic Radiology, Clinical Center of Kragujevac, Serbia in the period from January 2016 to September 2016. The radiological imaging was performed on the 64-slice MDCT scanner (Aquilion 64, Toshiba, Japan). All scans were performed in the axial plane, with subsequent multiplanar reconstruction (MPR). Due to the angulation of the sternal body coronal curved-planar, the images were obtained in order to show the whole length of the sternum and the vertical diameter of the sternal foramen. The scope of the thoracal scan examination was from thoracal inlet to the level of the adrenal glands, including the sternum in the field of scanning. The study included 422 patients (222 male and 200 female) aged from 21 to 80 years, without sternal deformities and pathological changes, which are referred to this examination for various reasons. The patients with sternal deformities, trauma or prior the sternal surgery, tumor changes of the sternum and younger than 20 years of age were excluded from the study. The patients who underwent chest imaging in the indicated period more than one time, were included in the study only once.

The MDCT examination was conducted in the native and post-contrast series, after intravenous injection of a contrast medium. The scanning parameters were: 120 kVp, mAs 90–120, gantry rotation of 0.75 sec, pitch 0.5 mm, slice thickness of 0.5 mm and 0.6–0.8 mm thickness reconstruction. The analysis of all images and MDCT data are performed on the Vitrea 2 workstation ver. 4.1.14.0 (Vital Images). All measurements were done by two independent radiologists, the experts in this field, using the commercially available software (Imaging Software ver. 4.1.14.0, Vital-Images). The measured values are given in millimeters. For the evaluation of inter-observer reliability, the intra-class correlation coefficient (ICC) was used and ICC > 0.8 was considered as an excellent agreement.

The obtained results were statistically analyzed according to gender. The analysis was done by using the statistical program (IBM SPSS Statistics 20) and included descriptive and analytical methods. Normality of data distribution was tested by the Kolmogorov-Smirnov test. The Student's *t*-test was used for a comparison of normally distributed data and the Mann-Whitney test was applied to the analysis of the variables which were not normally distributed. The level of a statistical significance was set at 0.05.

Results

The solitary foramen, located in the distal segment of the sternal body, was detected in 24 patients, representing 5.9% of the observed population (Figure 1). The presence of

the sternal foramen showed tendency of increasing with age. The average age in the group with detected foramen was 63.5 ± 7.5 years (63 ± 8.8 in the males and 64.3 ± 5.5 in the females). This developmental variant of sternum was usually oval-shaped and its transverse diameter varied in range from 1.7 to 10.6 mm. The mean width was 3.9 ± 1.9 mm, while the mean vertical diameter of the observed sternal foramen was 4.2 ± 1.7 mm and ranged from 2.5 to 8.4 mm. The sternal foramen was found in 14 males, representing 6.3% of male or 3.3% of all study participants. In females, this sternal variant was found in 10 of them, which was 5% of our female participants or 2.4% of whole study group. The males represented 58.3% and females 41.7% of the participants with the midline sternal foramen. The diameters of the detected sternal foramen in the males and females are given in Table 1.

The mean distance from the skin was 12.7 ± 3.3 mm (12.9 ± 3.4 in the males and 12.5 ± 3.4 in the females). The distance from skin to pericardium was on average 37.3 ± 8.2 mm in the whole observed population, with mean of 38.9 ± 8.9 mm in the males (ranged from 25.1 to 58.3 mm) and 35.1 ± 7 mm (ranged from 22.6 - 44.8 mm) in the females. In 6 males and 5 females, it was found that pleura was adjacent to the sternal foramen and the mean value of the distance from skin to pleura in the males was 25.7 ± 6 mm and 24.2 ± 6.4 mm in the females. The average value for both genders was 25 ± 5.9 mm.

The average depth of the foramen was 8.7 ± 2 mm, with the mean value of 8.5 ± 2.1 mm in the males and 8.9 ± 1.9 mm in the females. The mean distance from the posterior surface of foramen to pericardium was 12.7 ± 9.1 mm with a statistically significant gender difference as well as the distance from the skin to pericardium (Table 1).

Discussion

Importance of better knowledge of the anatomical variations has been pointed out among the anatomist and clinicians during the last decades. These structural fluctuations are often combined and ethnically different [6, 9, 10]. A good knowledge of human morphology is necessary for the proper interpretations of radiological images and diagnosis as well as planning of medical interventions. It is also important for the proper conclusions in pathology and forensic medicine [11].

The fluctuations of morphology of the anterior thoracic wall are not rare and are always interesting as potential clinical problems. They include the variations of sternum and the ribs as well as the joints of the thoracic wall caused by the aberrant development [9, 11].

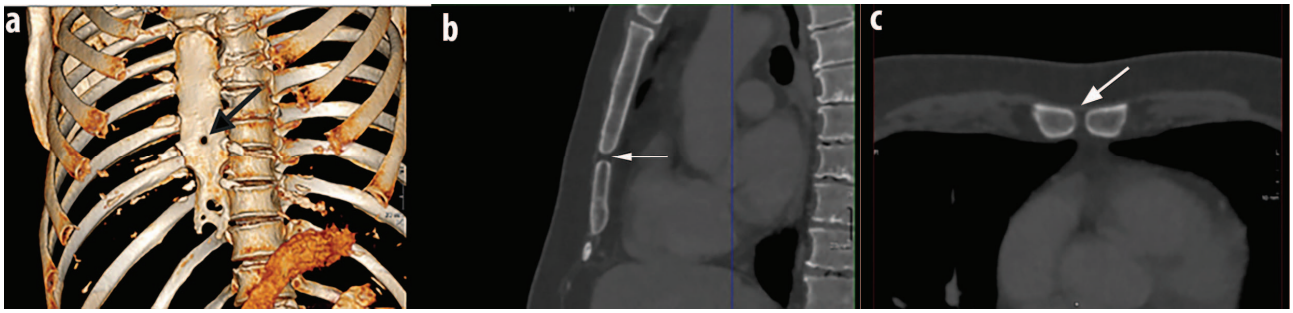

Fig. 1 – The foramen of the sternal body (marked by arrow): a) 3D reconstruction; b) multiplanar (MPR) sagittal reconstruction; c) MPR axial reconstruction.

Table 1

The morphometric characteristics of a single sternal body foramen (the measures are given in mm)

Foramen of the sternal body	Male	Female	Both gender
Transverse diameter, mean ± SD	4.0 ± 2.3	3.7 ± 1.2	3.9 ± 1.9 $p > 0.05$
Vertical diameter, mean ± SD	4.1 ± 1.9	4.3 ± 1.4	4.2 ± 1.7 $p > 0.05$
Foramen depth, mean ± SD	8.5 ± 2.1	8.9 ± 1.9	8.7 ± 2.0 $p > 0.05$
Skin – foramen distance, mean ± SD	12.9 ± 3.4	12.5 ± 3.4	12.7 ± 3.3 $p > 0.05$
Skin – pleura distance, mean ± SD	25.7 ± 6.0	24.2 ± 6.4	25.0 ± 5.9 $p > 0.05$
Skin – pericardium distance, mean ± SD	38.9 ± 8.9	35.1 ± 7.0	37.3 ± 8.2 $p < 0.05$*
Foramen (posterior surface) – Pericardium distance, mean ± SD	13.1 ± 8.9	10.3 ± 7.3	12.7 ± 9.1 $p < 0.05$*

***statistically significant gender difference; SD – standard deviation.**

Table 2

The comparison of the prevalence of midline sternal foramen to the findings of the previous studies and in the different populations

Study	Population	Sample size	Data / methodology	Prevalence of the midline sternal foramen		
				Male (% of male sample)	Female (% of female sample)	Total study sample (%)
Schratter et al. [16]	Austrian	100	CT	No data	No data	6
Cooper et al. [17]	USA	2016	Postmortem radiographs	68.9 (6.9)	31.1 (6.2)	6.7
Gossner [6]	German	352	CT	62.5	37.5	4.5
Paraskevas [18]	Greek	60	Dried sterna	100	/	5
Aktan and Savas [20]	Turkish	350	CT	63.2 (6.9)	36.8 (4)	5.4
		62	Dried sterna	No data	No data	3.2
Yekeler et al. [3]	Turkish	1000	MDCT	No data	No data	4.5
Babinski et al. [22]	Brazilian	180	Cadavers; Sceletons	93.75 No data	6.25 No data	16.6
Babinski et al. [23]	Brazilian	114	MDCT	58.3 (13.5)	41.7 (8.1)	10.5
Singh and Pathak [24]	Indian	343	Cadaveric	80.5 (13.1)	19.5 (8.8)	11.9
El-Busaid [4]	Kenyan	80	Dried sterna	No data	No data	11.2
Macaluso and Lucena [11]	Spanish	122	Postmortem radiographs	100 (6.1)	/	3.3
Ishii et al. [25]	Japanese	1053	MDCT	No data	No data	3.1
Present	Serbian	422	MDCT	58.3 (6.3)	41.7 (5)	5.9

CT – computed tomography; MDCT – multidetector computed tomography.

During the embryological development, the body of sternum consists of 4 segments (sternebrae) in the form of two cartilaginous sternal bars which migrate to the midline to be fused in cranio-caudal direction [2, 12]. The failure in fusing of the sternal bars or later ossification process results in defects such as foramina of the sternal body, manubrium or xiphoid processus, sternal clefts, or the combinations of these [3, 6, 13]. The sternal variations and aberrant morphology may have clinical implications regarding normal functions of internal organs. They also may be associated with heart and lung abnormalities [14]. The solitary perforations of the sternal body are common along the midline and are known as the sternal midline foramina [15].

Several earlier studies of different ethnic populations were focused on the prevalence and morphometry of the sternal midline foramina (Table 2). Similar to our results are those from the CT study conducted in Austria, with a diameter of the sternal body foramen ranging from 3 to 12 mm [16].

The prevalence of commonly single, round to oval sternal body foramen, with a diameter range from 3–10 mm, was slightly higher in a large cadaveric study conducted in the USA. In this study, this anatomic malformation was less frequent in the participants of white race (5.76%) and the hispanic population (5.84%) and with higher prevalence among the Afro-American population (9.01%). As expected, the most of these sternal variants are found in the elderly population group. The multiple sternal foramina were also described as well as the elongated ones [17].

The sternal body foramen was found on the CT scans in the German population [6] with lower prevalence in comparison to our study, while its mean width was similar (3.3 mm in the German population versus 3.9 mm in the Serbian population). It was emphasized that the practitioners may be aware of serious complications that may occur due to the presence of the sternal foramina and that the knowledge of the distance from the skin to pericardium may be helpful for a safe needle insertion, with conclusion that the sternal puncture with a needle inserted up to 2.5 cm is not life-threatening [6]. The average skin-pericardium distance that we found was greater than this, but the minimal one was 22.5 mm. The pericardium was adjacent to the foramen in 2 cases of our study (8.3%), while the lungs were adjacent in 45.8% cases (versus 53.3% in German study [6]) with skin-pleura distance ranging from 19.6 mm to 35.7 mm. The mediastinal fat adjacent to the sternal foramen was observed in 15 patients whose CT images we analyzed (62.5%), which is higher than in German population (33.33%) [6].

The slightly lower prevalence of midline sternal foramen was found in the Greek cadaveric study, while the diameters were similar to those that we observed. In their study sample, the number of sternal foramina was from one to five. The foramen of the sternal body was always single, while the multiple foramina were detected on the xiphoid processus [18]. However, the same authors in their earlier case report, described the sternal body foramen in a combination with the foramen of the xiphoid processus [19]. We did not find similar case in our study.

The CT study conducted in the Turkish population found the sternal midline foramen in both genders, with prevalence similar to one found in our population. Their

study also included the prepared sternal bones sample and the midline sternal foramen was found only in 2 of 62 cases [20]. In another Turkish study, the MDCT images were evaluated and the prevalence of the midline body foramen was lower. Among the other variants and defects of sternum, the foramen with a common localization in the distal half of the sternal body, had the mean diameter of 6.5 mm (ranging from 2 to 16 mm) which was higher than in other studies. In one case, it was assocciated with the sternal cleft in its continuation, while 73% of participants with the sternal body foramen had sclerotic band superior or inferior in its continuation [3]. A case of similar keywhole-shaped defect of sternum, as the foramen of the most distal part of the sternal body with continuation in the form of the xiphoid cleft, was described in a study of Saccheri et al. [21].

The analysis of cadaveric and dry sternal bones in Brazilians estimated the presence of the sternal midline foramen, with the highest prevalence in comparison to other studies, with the most common localization in the level of fourth costal notch of sternum. These foramina were solitary and round to oval in shape, as ones found in our study. The mean longitudinal and transverse diameter was also higher than we described [22]. Another study included MDCT images of the Brazilian patients of both genders and the sternal foramen was also highly prevailant, slightly more in females. The distribution among the gender groups was similar to our study while the mean distance and the range of distance from the skin to pericardium was equal [23].

The study conducted among the Indian population included the sternal samples of both genders, collected and analyzed postmortem, and revealed the high prevalence of the midline sternal foramen. It was higher in males than in females, with a tendency of increasing with age, which was also observed in our study, probably because of the increased muscle markings in the elderly [24]. The higher prevalence of the sternal body foramen with the common localization in the 5th intercostal segment was also found in the Kenyan population [4].

The percentage of the sternal body foramen that we found is higher in comparison with one found in a Spanish forensic study that described those foramina as solitary, size-variable, round to oval, located in the distal part of the sternal body that is similar to our findings [11]. Contrary to our observation, all cases with the sternal foramen were males. The lower prevalence was also found in the CT study conducted among the Japanese population, where the mean diameter was 5.3 mm [25].

Several case reports described the occurrence of the sternal midline foramen. Bermio and Hemalatha [2] described 5 × 6 mm sternal body foramen in the level of the junction of the third and fourth sternebrae. A case of a round, 18.75 × 12.50 mm large foramen of the distal half of a sternal body, found during autopsy, was also described by Jakhar et al. [14]. Another single large sternal foramen, with dimensions of 11.4 × 20.8 mm was described in a case report from India [26].

These variations are usually asymptomatic and observed during the radiological and forensic examinations or autopsy [19, 23]. However, they may be a serious risk factor for the severe complications that may occur during the sternal biopsy or acupuncture, considering the fact that the pleura, pericardium or great vessels are adjacent to the sternum [6, 23]. The bone marrow aspiration, that is often perform on sternum, may be complicated by unwanted needle penetration through the sternal foramen leading to pneumothorax, cardiac tamponade, or hemorrhage due to laceration of aorta [4–6, 21, 27–30]. According to some studies, in 80% of cases, the pneumothorax and cardiac tamponade could be a result of needle insertion through the sternal foramen [6, 18]. Although rare, infections of internal organs, were also reported as a complication of acupuncture, in the presence of the sternal foramina [23]. Regarding that, the biopsy of iliac crest, in order to perform a bone marrow aspiration, when possible, is considered to be safer. The clinicians must be aware of these unwanted possibilities, their asymptomatic character and difficult visibility on radiographs as well as the fact that these foramina are not palpable. Considering all above, the preventive ultrasound or CT images are recommended before the sternal biopsy or acupuncture procedures [6, 16, 31]. Also, these procedures should be performed in the proximal part of sternum, because common localization of the sternal foramina in the distal half of the sternal body [3].

Awareness of the possibility of the sternal variations is also important for the proper conclusions in forensic medicine or pathology, because these perforations could incorrectly be considered as gun wounds and injuries caused before death or the osteolytic lesion [20, 31].

Conclusion

The results presented in this paper confirmed the prevalence of 5.9% of the midline sternal foramen in the observed population, that is in accordance with the findings of the most similar studies conducted in another ethnic groups. The similarity is the most pronounced with those conducted in the Caucasian race, as expected. The existence of the anatomical sternal variations, usually asymptomatic, and its vicinity to the vital organs must not be ignored and the practitioners as well as forensics must be aware of such a possibility. Considering that, the preceding MDCT imaging could prevent serious complications of the sternal puncture.

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Differences and similarities between the symptoms and clinical signs in patients with pulmonary tuberculosis and pneumonia

Razlike i sličnosti u simptomima i kliničkim znacima bolesti među bolesnicima lečenim od tuberkuloze pluća i pneumonije

Sonja Smiljić*, Blagica Radović†, Aleksandra Ilić‡, Goran Trajković§, Sladjana Savić*, Zvezdan Milanović*, Milica Mijović**

**University of Priština/Kosovska Mitrovica, Faculty of Medicine,
*Department of Physiology, ‡Institute for Medical Statistics and Informatics,
**Department of Pathology, Kosovska Mitrovica, Serbia; Health Centre,
†Department of Pulomonology, Kosovska Mitrovica, Serbia; University of Belgrade,
Faculty of Medicine, §Institute for Medical Statistics and Informatics, Belgrade, Serbia**

Abstract

Background/Aim. Tuberculosis in the second decade of the 21st century is an infectious disease with the highest mortality rate. In addition, in developed countries, pneumonia is the major couse of morbidity and mortality in adults. The aim of our study was to point out the differences and similarities between symptoms, laboratory parameters and clinical indicators in patients with pulmonary tuberculosis (PTB) and patients with pneumonia in the general population and in people belonging to the high risk groups for developing tuberculosis. **Methods.** This prospective study included patients with PTB (n = 70) and pneumonia (n = 75) treated at the Pulmonology Department of Clinical Hospital Center in Kosovska Mitrovica. **Results.** PTB was more frequent in men, 30–39 years of age (OR; 6:08), mainly from rural areas (p = 0.001), and with lower levels of education (p = 0.031). Pneumonia was more frequent in women older than 60 years of age (p = 0.0012). Night sweats (p = 0.001) and weight loss (p = 0.062) were significantly more frequent in patients with PTB, while chest pain (p = 0.001) and high temperature (p = 0.036) were more common in patients with pneumonia. X-ray changes in patients with PTB were located in the upper fields (p = 0.001), or appeared to be bilateral (p = 0.004). The strongest predictor associated with an increased risk of night sweats was diagnosed PTB (OR = 30.0). The chest pain was a predictor of pneumonia, unilateral changes (OR = 4.65) in the lower lung fields (OR = 0.08). **Conclusion.** Night sweats, weight loss and chest X-ray abnormalities in upper fields were significant indicators of PTB. Chest pain, fever and chest X-ray abnormalities in lower fields were significant indicators of pneumonia.

Key words:
tuberculosis, pulmonary; pneumonia; signs and symptoms; risk factors.

Apstrakt

Uvod/Cilj. Tuberkuloza je u drugoj dekadi 21. veka infektivna bolest sa najvišom stopom mortaliteta. Uz to, u razvijenim zemljama, pneumonija je glavni uzrok morbiditeta i mortaliteta odraslih osoba. Cilj našeg istraživanja bio je da se ukaže na razlike i sličnosti u simptomima, laboratorijskim parametrima i kliničkim pokazateljima u ranoj dijagnozi tuberkuloze u opštoj populaciji i kod osoba koje pripadaju rizičnim grupama za oboljevanje od tuberkuloze pluća. **Metode.** Prospektivnom studijom smo obuhvatili sve obolele od tuberkuloze pluća (n = 70) i pneumonije (n = 75) lečene na Odeljenju pulmologije Kliničkobolničkog centra u Kosovskoj Mitrovici. **Rezultati.** Od tuberkuloze pluća češće su obolevali muškarci, starosti 30–39 godina (OR 6.08, 95 CI% 1,16–31,84), uglavnom sa sela (p = 0,001), nižeg stepena obrazovanja (p = 0,031). Od pneumonije su češće obolevale ženske osobe starije od 60 godina (p = 0,012). Kod obolelih od tuberkuloze pluća bilo je značajno češće noćno znojenje (p = 0,001) i gubitak težine (p = 0,062), kod obolelih od pneumonije bol u grudima (p = 0,001) i visoka temperatura (p = 0,036). Radiografske promene kod obolelih od tuberkuloze pluća bile su uglavnom u gornjim poljima pluća (p = 0,001) i obostrano (p = 0,004). Najjači prediktor povezan sa dijagnozom tuberkuloze je noćno znojenje (OR = 30,0). Bol u grudima je bio prediktor pneumonije i bilateralnih promena (OR = 4,65) u donjim plućnim poljima (OR = 0,08). **Zaključak.** Noćno znojenje, gubitak težine i radiografske promene u gornjim poljima pluća bile su značajni indicatori tuberkuloze pluća. Bol u grudima, visoka tempeartura i ra-

Correspondence to: Sonja Smiljić, University of Priština/Kosovska Mitrovica, Faculty of Medicine, Anri Dinana bb, 38 220 Kosovska Mitrovica, Serbia. E-mail: sonja.smiljic@med.pr.ac.rs

diografske promene u donjim poljima pluća bile su značajno češće kod pneumonije.

Ključne reči:
tuberkuloza pluća; pneumonija; znaci i simptomi; faktori rizika.

Introduction

Tuberculosis (TB) in the second decade of the 21st century is an infectious disease with the highest mortality rate. During 2015, TB affected 9.4 million people, with a fatal outcome in 1.4 million patients that were treated. There has been a decline in incidence in most areas around the world, but the scale of this decline is far smaller than expected. The current global TB incidence rate continues to fall by around 2% per year which is insufficient to achieve the goal of eliminating TB by year 2050 [1]. The existing recommendations for an early detection of TB appear to be insufficient. This is particularly true for the high-risk groups with clearly expressed symptoms that do not seek medical advice. The potential health, social and/or economic benefits, would probably be higher if not for the delayed diagnosis in the high-risk groups [2]. Particular attention should be paid to people with bad life habits (cigarette smoking, alcohol consumption) and the high-risk groups: HIV positive, diabetics, refugees, prisoners, the homeless and the elderly. For any given risk group, an early treatment provides less severe clinical manifestations and less economic costs, and in epidemiological sense, prevents the spread of the epidemic [3].

The diagnosis of pulmonary TB (PTB) is based on the presence of respiratory symptoms and characteristic signs, laboratory parameters, a direct microscopy examination of sputum, sputum culture and chest radiography [4]. In countries with a limited health infrastructure, the diagnosis of PTB, and especially a sputum smear-negative PTB (SNPT), can present a challenge [5]. Symptom screening is a key component in fight against TB and is one of the main strategies for the eradication of this contagious disease. The significant symptoms include a prolonged cough that lasts more than 2 weeks, a productive cough, hemoptysis, fever, night sweats, weight loss and chest pain [6]. Symptom screening is appealing because it is simple, does not require expensive equipment and can be used in general medical practice. Cough is the main symptom of TB and is considered a positive symptom if it lasts longer than two weeks. It could be accompanied by sputum production or haemoptysis [7]. Productive cough is more common in sputum positive TB and is associated with 4–5 times higher level of disease transmission compared with sputum negative TB [5]. Risk factors for developing PTB are malnutrition and low body mass index (BMI) which make symptoms such as weight loss and poor appetite particularly important [8]. TB patients report night sweats as one of the characteristic symptoms [9], while chest pain is rarely reported [10]. However, it is necessary to confirm the diagnosis in a fast and systematic way as these signs are also indicative of a number of other respiratory diseases.

Respiratory diseases such as PTB and pneumonia induce a series of laboratory abnormalities such as anemia and accelerated erythrocyte sedimentation rate. Numerous studies documented anemia in patients with PTB. Anemia, caused by chronic infections such as TB, results from the suppression of erythropoiesis by inflammatory mediators [11, 12].

Symptom screening is the first step in diagnosing PTB, although the symptoms themselves have relatively little significance. Detecting suspicious symptoms provides a timely suspicion of disease while chest radiography, culture and/or findings of sputum positive for Koch bacillus will lead to a definite diagnosis. A direct microscopy examination of sputum should be a routinely applied analysis in all patients who have manifested symptoms of PTB. Late diagnosis of TB largely influences the low rate of incidence decline and leads to poorer medical outcomes [13].

In developed countries, pneumonia is the major cause of morbidity and mortality in adults [14] and it leads to a high level of hospitalization, especially in the elderly. The onset is sudden, accompanied by the characteristic respiratory symptoms. Lifestyle factors (cigarette smoking, alcohol abuse, social determinants) and associated diseases (cardiovascular diseases, diabetes mellitus, HIV) are high risk factors for developing TB and pneumonia. Patients can also exhibit multiple risk factors at the same time [15].

The aim of our study was to point out the differences and similarities between symptoms, laboratory parameters and clinical indicators in patients with PTB and patients with pneumonia in the general population and in people belonging to the high risk groups for developing TB.

Methods

The survey was conducted in accordance with the ethical principles and was approved by the Ethics Committee of the Faculty of Medicine, University of Priština, with a temporary seat in Kosovska Mitrovica.

A prospective study was conducted at the Department of Pulmonology of the Health Center in Kosovska Mitrovica, the reference hospital for TB treatment. The study included patients with TB and pneumonia treated in the period between 2011 and 2015. All hospitalized patients (145) were divided into 2 groups: 70 patients with PTB and 75 patients with pneumonia.

On admission, the patients' data regarding demographics, age, gender, residence, marital status, education, employment status and social determinants was gathered. We also processed risk factors for developing TB and pneumonia, including smoking, alcohol consumption, drug use, prolonged use of corticosteroids, the use of immunosuppressive therapy and comorbidities such as diabetes mellitus, chronic renal failure, cancer, chronic obstructive pulmonary disease, liver cirrhosis, congestive heart failure and HIV infection.

All of the patients exhibited positive symptoms of cough, expectoration, haemoptysis, chest pain, fever, night

sweats and asthenia. Clinical signs of the disease included weight loss, anemia and high blood sedimentation rate. Weight loss was defined as a positive symptom if it exceeded 10% of the total body weight in the last three months. Hemoglobin below 12 g/dL in women and 13 g/dL in men was taken as the reference value for the confirmation of anemia. Haemoptysis were positive if it occurred only once.

Clinical follow-up included an examination of the sputum before the treatment (negative and positive results), the type of TB (new or relapse) and the outcome of the treatment. The results of chest X-rays were categorized according to the scale of the changes, their localization and their morphological structure.

Sputum samples were taken from all the patients for a direct microscopy of the preparations stained according to the Ziehl-Neelsen method. Also, a cultivation of bacillus on Lowenstein-Jansen medium was performed for all samples. Sputum was collected in the morning, before eating, after a spontaneous expectoration. Each sputum positive for direct microscopy was verified by the culture on Löwenstein-Jansen medium. PTB was bacteriologically confirmed if the two sputum findings confirmed bacillus and/or in a case of positive sputum cultivation. The final diagnosis of pulmonary TB was made based on the M+ in sputum and/or chest X-rays. The diagnosis of pneumonia was made based on clinical findings, bacteriological sputum findings and chest X-rays. The interpretation of chest X ray abnormalities was performed by a radiologist. Chest postero-anterior X-rays in both groups focused on pulmonary parenchyma and caverns. The interpretation of abnormalities in the pulmonary parenchyma included unilateral or bilateral changes and location changes in the lower, medium and upper fields.

Statistical analysis

The data were analyzed by the descriptive statistical methods and presented as frequencies and relative numbers. For the analysis of frequency differences between the groups, the chi-square test was used. Binary logistic regression was the technique used to analyze the dependencies between activities. The multiple logistic regression model included all the predictors that had statistical significance at 0.05 in the single logistic regression model. The criterion for a statistical significance was $p < 0.05$.

For the statistical data analysis we used the SPSS Statistics 22 software program SPSS Statistics 22. Inc., Chicago, IL, USA).

Results

The study included the TB and pneumonia patients treated at the Pulmonology Department in the period from 2011 to 2015. Out of 145 patients, 70 were treated for PTB and 75 for pneumonia. There were significant differences in demographic characteristics between the two groups of patients. There was a significantly higher incidence of PTB in the males ($p < 0.001$), middle-aged and older, while the patients suffering from pneumonia were older than 60 years of age ($p = 0.0012$). In relation to their place of residence, patients from the rural areas were significantly more prone to TB infection ($p < 0.001$). Family status had no significant influence on the onset of these two diseases. The TB patients belonged to lower-educational level groups ($p = 0.025$). Patients' occupation did not influence the onset of these respiratory infectious diseases and there was no significant difference of incidence between the patients with TB and the patients with pneumonia ($p = 0.394$) (Table 1).

Lifestyle and comorbidity impact the morbidity of both TB and pneumonia. In the patients with the TB bad habits, such as cigarette smoking ($p = 0.002$) and alcohol consumption ($p = 0.050$), were dominant. Associated diseases were equally present in both groups of patients, except for diabetes mellitus which was significantly more frequent in the patients with pneumonia ($p = 0.024$). The social determinants were important in the patients with PTB ($p = 0.001$) (Table 1).

The respiratory symptoms were characteristic for both groups of patients. The onset of the symptoms in the patients suffering from TB was longer than 2 weeks before visiting a doctor ($p < 0.001$). Cough, expectoration, hemoptysis and fatigue symptoms did not differ significantly. The patients with TB frequently reported weight loss and night sweats ($p < 0.001$). In the patients with pneumonia, chest pain was prevalent ($p < 0.001$) as well as a high temperature ($p = 0.036$). The laboratory variables, hemoglobin values, hematocrit and erythrocyte sedimentation rate did not differ significantly between the two groups of patients. Positive bacteriological culture of sputum also did not differ (Table 2).

There were significant differences in radiographic changes between the groups. In the patients with the TB X-ray changes were more common in the upper lung fields ($p < 0.001$) and both lungs were significantly more likely to have been affected by the changes ($p = 0.004$). The incidence of a relapse in the patients with TB was 7%, while in the patients with pneumonia there was no relapse recorded. The relapse of the disease was significantly more frequent in PTB ($p = 0.018$). The hospital treatment of the patients with TB was significantly longer than in the patients with pneumonia ($p < 0.001$) (Table 2).

Night sweats were typical and statistically highly significant symptom in the patients with TB. In the simple logistic models, the variables associated with an increased risk of developing night sweats were: the PTB diagnosis ($p = 0.001$), 30–39 years of age ($p = 0.001$) and 40–49 years of age ($p = 0.004$) compared to the over-60 years of age group taken as the reference value, life in the city ($p = 0.022$) and diabetes mellitus ($p = 0.009$). The variables associated with a reduced risk of developing night sweats are the X-ray changes in the upper ($B = -1.77$; $p = 0.022$) and middle lung fields ($B = -2.60$; $p = 0.007$). In the multiple logistic regression model, the strongest predictor associated with an increased risk of night sweats was a diagnosed PTB ($p < 0.001$; OR = 30.0 (6.56–137.0) which indicated that those suffering from PTB had 30 times higher risk of developing night sweats (Table 3).

Chest pain is an important symptom in the patients with pneumonia. The univariate and multivariate logistic regres-

sion model indicated that chest pain was not a predictor for developing PTB (p = 0.002; OR = 0.006 (0.009–0.35). Also, there was no significant relation between chest pain as an important symptom of pneumonia and smoking habits (p = 0.05; OR = 0.30 (0.09–1.0). The pain predictors were unilateral changes (p = 0.028; OR = 4.65 (1:19 to 18:21) in the lower lung fields (p = 0.003; OR = 0.08 (0:02 to 0:41) (Table 4).

Table 1

Sociodemographic characteristics and risk factors in the patients with pulmonary tuberculosis (PTB) and the patients with pneumonia

Characteristics	PTB (n = 70)	Pneumonia (n = 75)	*p*
	n (%)	n (%)	
Age (years)			
20–29	11 (15.7)	11 (14.7)	
30–39	6 (8.6)	7 (9.3)	
40–49	10 (14.3)	16 (21.3)	0.0012
50–59	26 (37.1)	7 (9.3)	
> 60	17 (24.3)	34 (45.3)	
Sex			
male	49 (70.0)	30 (40.0)	<0.001
female	21 (30.0)	45 (60.0)	
Residence			
rural	57 (81.4)	36 (48.0)	<0.001
urban	13 (18.6)	39 (52.0)	
Marital status			
single	30 (42.9)	28 (37.3)	0.497
married	40 (57.1)	47 (62.7)	
Education			
primary	41 (58.6)	30 (40.0)	
secondary	29 (41.4)	42 (56.0)	0.031
high	0 (0)	3 (4.0)	
Employment status			
unemployed	15 (21.4)	22 (29.3)	
toiler	20 (28.6)	13 (17.3)	0.394
office worker	11 (15.7)	13 (17.3)	
pensioner	24 (34.3)	27 (36.0)	
Smoking status			
never	23 (32.9)	43 (57.3)	
current	39 (55.7)	20 (26.7)	0.002
former	8 (11.4)	12 (16.0)	
Alcohol use	12 (17.1)	5 (6.7)	0.050
Social determinants	29 (41.4)	9 (12.0)	< 0.001
Cardiovascular diseases	15 (21.4)	23 (30.7)	0.206
Diabetes mellitus	9 (12.9)	21 (28.0)	0.024
COPD	11 (15.7)	8 (10.7)	0.368

COPD – chronic obstructive pulmonary disease.

Table 2

Frequency of reported symptoms, clinical signs and radiological findings in the patients with pulmonary tuberculosis (PTB) and the patients with pneumonia

Parameters	PTB (n = 70)	Pneumonia (n = 75)	*p*
	n (%)	n (%)	
Simptom duration (weeks)			
< 2	11 (15.7)	53 (70.7)	< 0.001
> 2	59 (84.3)	22 (29.3)	
Cough	58 (82.9)	66 (88)	0.379
Productive cough	42 (60)	41 (54.7)	0.517
Hemoptysis	8 (11.4)	9 (12.0)	0.915
Chest pain	8 (11.4)	46 (61.3)	< 0.001
Fever	44 (62.9)	59 (78.7)	0.036
Night sweats	42(60.0	14 (18.7)	< 0.001
Asthenia	43 (61.4)	48 (64)	0.749
Weight loss	35 (50.0)	26 (34.7)	0.062
Anemia	22 (31.4)	18 (24.0)	0.317
Sedimentation rate	58 (82.9)	65 (86.7)	0.523
Culture			
negative	28 (40.0)	38 (50.7)	
positive	42 (60.0)	37 (49.3)	0.197
Location CXR abnormality			
upper field	54 (77.2)	1 (1.4)	
medium field	8 (11.4)	46 (61.3)	< 0.001
lower field	8 (11.4)	28 (37.3)	
Any CXR abnormality			
right	9 (12.8)	26 (33.3)	
left	24 (34.3)	25 (34.7)	0.004
bilateral	37 (52.9)	24 (32.0)	
Radiological severity			
initial	37 (52.9)	49 (65.3)	0.299
advanced TB	33 (47.1)	26 (34.7)	
Outcomes			
cured	65 (92.9)	75 (100.0)	0.018
retreatment	5 (7.1)	0 (0)	
Intra hospital therapy (days)			
< 30	17 (24.3)	70 (93.3)	
< 60	21 (30.0)	5 (6.7)	< 0.001
> 60	32 (45.7)	0 (0)	

CXR – chest x-ray.

Table 3

Uni- and multivariate logistic analysis of the association of night sweat and risk factor and clinical characteristics

Factors	Univariate OR (95% CI)	*p*	Multivariate OR (95% CI)	*p*
Diagnosis				
PTB	34.4 (4.07–290.9	0.001	30.0 (6.56–137.0)	< 0.001
pneumonia	Reference			
Age (years)				
20–29	15.0 (2.26–100.1)	0.005	5.51 (1.48–20.46	0.011
30–39	64.0 (5.1–798.9)	0.001	15.79 (2.67–93.54	0.002
40–49	14.8 (2.35–93.64)	0.004	6.18 (1.64–23.33)	0.007
50–59	9.0 (1.78–45.09)	0.008	4.47 (1.33–15.02)	0.016
> 60	Reference			
Sex	1.11 (0.35–3.55)	0.86		
Residence				
rural	Reference			
urban	4.30 (1.23–14.98)	0.022		
Education level				
primary	Reference			
secondary	0.29 (0.08–1.02)	0.054		
high	0 (0.0)	0.999		
Smoking status				
no	Reference			
yes	0.91 (0.27–3.06)	0.872		
former	1.63 (0.36–7.49)	0.528		
Any alcohol use	0.24 (0.03–1.84)	0.169		
Social determinants	0.70 (0.19–2.67)	0.606		
Diabetes Mellitus	8.59 (1.73–42.65)	0.009	4.80 (1.34–17.21	0.016
Cough duration				
< 2 weeks	Reference			
> 2 weeks	1.12 (0.34–3.70)	0.858		
Fever	1.52 (0.45–5.12)	0.499		
Location CXR abnormality				
upper field	0.17 (0.04–0.77)	0.022	0.17 (0.04–0.82)	0.027
medium filed	0.07 (0.01–0.49)	0.007	0.28 (0.08–0.98)	0.047
lower field	Reference			
Any CXR abnormality				
right	Reference			
left	2.19 (0.63–7.59)	0.218		
bilateral	2.85 (0.74–11.02	0.128		
Outcomes	8.62(0.59–126.6)	0.116		
Intra hospital therapy (days)				
< 30	Reference			
< 60	0.68 (0.14–3.22	0.623		
> 60	4.23 (0.72–24.7)	0.109		

OR – odds ratio; CI – confidence interval; PTB – pulmonary tuberculosis; CXR – chest x-ray.

Table 4

Uni- and multivariate logistic analysis of the association of chest pain and risk factor and clinical characteristics

Factors	Univariate	*p*	Multivariate	*p*
	OR (95% CI)		OR (95% CI)	
Diagnosis				
PTB	0.01 (0.001–0.15)	0.001	0.06 (0.009–0.35)	0.002
pneumonia	Reference			
Age (years)				
20–29	1.79 (0.266– 12,025)	0.549		
30–39	4.37 (0.422–45,251)	0.216		
40–49	2.06 (0.344–12,785)	0.435		
50–59	2.06 (0.376–11,314)	0.404		
> 60	Reference			
Sex	0.66 (0.231–1,937)	0.459		
Residence				
rural	Reference			
urban	1.45 (0.449–4,713)	0.532		
Education level				
primary	Reference			
secondary	0 (0.0)	0.999		
high	1.81 (0.501–6,546)	0.366		
Smoking status				
no	Reference			
yes	0.30 (0.09–1.0)	0.050	0.39 (0.15–1.0)	0.050
former	0.11 (0.02–0.68)	0.018	0.13 (0.03–0.55)	0.006
Any alcohol use	0.95 (0.130–7,036)	0.964		
Social determinants	2.17 (0.491–9,650)	0.306		
Diabetes mellitus	0.701 (0.168–2,933)	0.627		
Cough duration				
< 2 weeks	Reference			
> 2 weeks	2.45 (0.742–8,140)	0.141		
Fever	0.62 (0.181–2,171)	0.461		
Location CXR abnormality				
upper field	0.23 (0.029–1,921)	0.177	0.38 (0.07–2.03)	0.255
medium field	0.18 (0.05–0.71)	0.014	0.45 (0.15–1.33)	0.148
lower field	Reference			
Any CXR abnormality				
right	Reference			
left	4.65 (1.19–18.21)	0.028	2.53 (0.83–7.73)	0.102
bilateral	1.83 (0.459–7,362)	0.390	1.14 (0.37–3.49)	0.819
Outcomes	1.56 (0.068–35,668)	0.781		
Intra hospital therapy (days)				
< 30	Reference			
< 60	7.13 (1.07–47.64)	0.043		
> 60	4.14 (0.360–47,607)	0.254		

OR – odds ratio; CI – confidence interval; PTB – pulmonary tuberculosis; CXR – chest x-ray.

Discussion

The diagnosis of TB can be established through examining clinical symptoms [1], chest radiography [3], sputum culture, sputum microscopy and the combinations of these [6]. In our study, we evaluated the symptoms, laboratory parameters and clinical signs of the disease in the patients with PTB and pneumonia in Northern Kosovo. These can contribute to determining the diagnostic value of symptoms and provide the support for improving strategies for the detection and diagnosis of new cases of PTB. Symptom screening contributes to an early detection and reduces the spread of PTB [3].

Anti-TB dispensary (ATD) in Kosovska Mitrovica is a very well organized medical institution for a complete diagnosis of TB. Most patients with suspected symptoms are referred to ATD, where the diagnosis is quickly given after a direct microscopic examination of sputum for Koch's bacillus and the chest X-ray. Due to similar initial symptoms of pneumonia and TB, it can happen that a general practitioner does not recognize TB which postpones anti-TB treatment, and such a patient becomes potentially contagious to the environment and can expect a worse outcome.

One of the major causes of morbidity and mortality in adults in developed countries is pneumonia [13]. It is, also, a frequent cause of hospitalization in the elderly. The main risk factors for the incidence of pneumonia, besides older age, are associated diseases. In people older than 60 years of age, the most common comorbidities are diabetes mellitus [16], a metabolic syndrome, cardiovascular diseases and chronic obstructive pulmonary diseases [13]. There is a high prevalence of pneumonia in patients with multiple lifestyle risk factors and comorbidities. Pneumonia often affects older men while, in our study, the women were more likely to be afflicted (60%) [17].

Several lifestyle factors are associated with an increased risk of PTB and pneumonia, including smoking and alcohol abuse [13, 14]. Smoking and excessive alcohol abuse are major health risks globally and are targets for interventions to reduce the global burden of this disease. Ensuring that patients make appropriate lifestyle changes would help reduce the overall burden of pneumonia. Similarly, being underweight may predispose patients to pneumonia due to the consequences of the undernutrition conditions on immune function, so assessment of the nutritional status of vulnerable patients might help identify those at increased risk of PTB and pneumonia [10, 17, 18].

The risk factors for PTB are: male gender, low BMI and alcohol consumption [19, 20]. The survey of 14 countries with the highest rate of TB incidence showed that the risk factors are more common in men than in women. Men of lower education, middle aged and older, were more likely to develop PTB. Also, men who consume alcohol and smoke cigarettes were significantly more predisposed to develop TB. There were less smokers among the affected women and they rarely consumed alcohol [17].

High-risk groups include people with a significantly higher incidence and prevalence of TB than the general population. They may be people with an individual risk of morbidity (such as HIV infection), or people from specific geographical locations or institutions. Smokers, alcohol consumers, diabetics or people with a BMI < 18.5 kg/m^2 are independently associated with the risk of developing TB. In most of the developed countries, diabetes is associated with a high BMI, where an associated obesity may be the cause of diabetes. Obesity and diabetes have a high prevalence in developed countries, and their possible interaction with smoking or heavy alcoholism in developing an active TB is a cause for worry [21, 22].

Smoking, alcohol consumption, diabetes and a low BMI can lead to a progression from a latent to an active form of TB. Possible mechanisms for smoking include the impaired clearance of secretions on the tracheobronchial mucosal surface, reduced phagocytic function of pulmonary alveolar macrophages, decreased production of tumor necrosis factor in pulmonary macrophages and increased iron overload in pulmonary macrophages [18, 23]. Chronic alcohol use has been shown to reduce a macrophage response and activate the immune system thus raising the risk of morbidity. The experimental studies showed that hyperglycemia may affect a host's immune response to the PTB. Malnutrition can reduce the host's protective immune response either by interfering in the interaction between monocyte-macrophages and T-lymphocytes and their cytokines, or by secondary immunodeficiency that increases the host's susceptibility to infection.

The important symptoms in the diagnosis of TB are: cough that persists for at least 2 weeks, expectoration, fever, night sweats, weight loss, asthenia, chest pain, and hemoptysis [24]. There is a possibility of only one symptom being present or a combination of several sensitive symptoms associated with TB. Cough is the main symptom of TB but also the main cause of the transmission of this disease. It occurs as a consequence of an inflammatory response to mycobacterial infection. An adequate response to therapy is manifested by the reduction of cough [7]. A cough that lasted for more than 2 weeks was present in 82.9% of our patients suffering from TB, while in 60% of the cases it was accompanied by sputum production, which coincides with the data of other studies [8, 12]. A cough that lasted longer than 2 weeks was significantly more frequent among sputum-positive TB [25], while a cough that lasts less than 2 weeks can be symptomatic of SNPT, but WHO recommends including a cough of any duration in the assessment of TB [24]. Early diagnosis and treatment of sputum-positive PTB (SPPT) in patients with a chronic cough is of high priority in reducing the transmission of TB [26, 27].

However, cough was equally frequent in patients with PTB and pneumonia. In our patients suffering from PTB, the symptoms started more than 2 weeks prior to visiting a doctor and they were manifested gradually, first with a cough and sputum production, and later with night sweats (60%), subfebrile temperature (62.9%) and weight loss (50%). In our patients suffering from pneumonia, the symptoms were sudden and fast developing and most of the patients were hospitalized in less than 2 weeks from the onset of symptoms. The patients suffering from pneumonia experienced a high temperature (78.7%) and chest pain that intensified during breathing (61.3%).

The symptoms that were particularly significant in patients with TB are night sweats and weight loss which, together with a persistent cough increase the specificity of these symptoms. Chest pain during breathing (11.4%) is not a significant symptom in the diagnosis of PTB [15]. Chest pain is one of the most common symptoms in the general population and can be the result of chest, abdomen and internal organs related diseases. One of the more common causes of chest pain are respiratory diseases, especially pneumonia [10]. Symptom screening is simple and is used in general medical practice. However, compared to the symptom screening, the chest radiography shows greater accuracy, while the combination of the two provides a far greater reliability [28]. Poor performance of symptom screening in the PTB detection was recorded in several studies, including the symptoms with the highest sensitivity, such as cough, fever, night sweats and weight loss [26].

Particular attention should be paid to the diagnosis of sputum negative TB. In areas with a higher prevalence of TB and HIV, the clinical signs and inexpensive tests, such as direct microscopy, tuberculin skin test and chest radiography, are of great importance in the diagnosis of PTB [29, 30]. Anorexia, asthenia and a less persistent cough are good predictors of SNPT. These symptoms thus deserve to be recommended as indicators of the SNPT diagnosis within an ATB dispensary. This strategy can help reduce morbidity and mortality associated with a late SNPT diagnosis [30]. Sputum-negative TB was present in 40% of those affected in Northern Kosovo, which is consistent with the results of other studies [5, 15, 30, 31], while the atypical radiographic changes occurred in 23% of the affected. The symptom screening had a significant role in these cases as it enabled the initial treatment until the disease was finally confirmed by Löwenstein-Jensen culture medium. Culture is the golden standard for laboratory confirmation of PTB [32].

PTB and pneumonia induce a series of laboratory abnormalities such as anemia, accelerated erythrocyte sedimentation rate, low serum albumin levels, hyponatremia, abnormal liver function, leukocytosis and hypocalcaemia. Numerous studies documented anemia in patients with TB. Anemia was present in 31.9% of our patients, but in most cases it was benign. Anemia, caused by chronic infections such as TB, results from the suppression of erythropoiesis by inflammatory mediators [33]. On the other hand, the disruption of iron homeostasis occurs with an increased absorption and retention of iron in the reticuloendothelial system in chronic infections such as TB [11]. Iron is an important growth factor. *Mycobacterium tuberculosis* and the retention of iron in the reticuloendothelial system are seen as defense mechanisms. Anemia improves with sputum conversion. Female gender and older age are risk factors for the concurrence of TB and anemia. Anemia is a common hematological abnormality in patients with PTB, it is usually mild and improves with the anti-TB treatment [34].

The symptom screening is the first step in early diagnosis of PTB both in the general population and high-risk groups. Patients who report symptoms indicating PTB should be referred to the microbiologic examination of sputum taken from three successive samples, sputum culture and chest X-ray. The confirmation of negative sputum smear results and the radiographic changes uncharacteristic of PTB further complicate an early diagnosis. In our patients that were treated for PTB night sweats were one of the characteristic symptoms which indicated 30 times greater risk of developing PTB; however, the radiographic changes were not in correlation with this symptom of the disease.

The univariate and multivariate logistic regression indicated chest pain as a significant predictor of pneumonia, accompanied by radiographic changes on the left or the right side and in the lower lung fields, which facilitated the diagnosis of pneumonia. Some symptoms in patients with PTB and pneumonia overlap and result in a late diagnosis of PTB. It is necessary to pay attention to the characteristic symptoms and clinical signs of the disease especially among high-risk groups, and refer the affected to appropriate centers for further diagnostic procedures. The bacteriological confirmation of sputum and chest radiography are important in the confirmation of suspected PTB. Early diagnosis of the disease is one of the first steps in its suppression.

Conclusion

TB incidence was more frequent in middle-aged and elderly men with bad life habits, smokers and alcohol abusers, people of low education and social status. Symptomatic cough lasted for more than 2 weeks before being reported to a doctor. The most pronounced symptoms were night sweats and weight loss. Patients treated for pneumonia at the same time were more frequently women, older than 60 years of age. Pneumonia and diabetes mellitus comorbidities were significant. The affected visited a doctor in less than 2 weeks from the onset of symptoms of the disease. A significantly higher number of patients with pneumonia had a high fever and chest pain during breathing.

The strongest predictor associated with an increased risk of night sweats was PTB. High fever and chest pain during breathing were correlated with the radiographic changes in middle or lower lung fields which indicated pneumonia. In conclusion, this study may help physicians understand the link between the symptoms, their duration, the risk factors and radiological findings in early diagnosis of PTB.

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Translation to Serbian, cultural adaptation, reliability testing and validation of the questionnaire estimating the fear of injections

Prevođenje na srpski jezik, transkulturalna adaptacija, ispitivanje pouzdanosti i validacija upitnika za procenu straha od injekcija

Dejan Z. Aleksić*, Miloš N. Milosavljević†, Andriana M. Bukonjić†, Jasmina R. Milovanović‡, Zoran M. Protrka§, Vesela B. Radonjić‡, Slobodan M. Janković‡, Srdjan M. Stefanović‡

University of Kragujevac, Faculty of Medical Sciences, *Department of Neurology, †Department of Pharmacy, ‡Department of Pharmacology and Toxicology, §Department of Gynecology and Obstetrics, Kragujevac, Serbia

Abstract

Background/Aim. The two-part questionnaire called Injection Phobia Scale (IPS)-Anxiety and IPS-Avoidance represents one of the most commonly used questionnaires for assessing the fear of injections. The aim of the present study was to translate and culturally adapt this questionnaire from English into Serbian as well as to assess reliability and validity of the translation. **Methods.** The translation and cultural adaptation of the IPS–Anxiety and IPS–Avoidance was performed in accordance with the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) guidelines. Reliability testing, factor analysis and validation of Serbian translation of IPS-Anxiety and IPS-Avoidance were carried out on a sample of 485 students of pharmacy, or medicine at the University of Kragujevac, Serbia. **Results.** Serbian translation of IPS-Anxiety and IPS-Avoidance demonstrated high internal consistency with Cronbach's alpha of 0.934 for IPS-Anxiety and 0.911 for IPS-Avoidance. Factor analysis of IPS-Anxiety showed that there are two domains, which we have called as Direct Experience (9 items) and Indirect Experience (9 items); factor analysis of IPS-Avoidance also pointed out on two domains referring to direct and indirect fear of injections. Female students scored higher on the scale showing more extensive injection phobia than male students. It is also interesting that students of pharmacy have higher level of injection phobia than students of medicine, and those students of the fifth year of study feel more fear of injections than students from the first four years. **Conclusion.** Serbian translation of IPS-Anxiety and IPS-Avoidance showed good psychometric properties on population consisted of students medicine and pharmacy.

Key words:
fear; injections; surveys and questionnaires; translating; serbia.

Apstrakt

Uvod/Cilj. Upitnik sastavljen iz dva dela pod nazivom *Injection Phobia Scale* (IPS)-*Anxiety* i IPS-*Avoidance* predstavlja jedan od najčešće korišćenih upitnika za procenu straha od injekcija. Cilj ove studije bio je da se ovaj upitnik prevede i transkulturalno prilagodi sa engleskog na srpski jezik, kao i da se proceni pouzdanost i validnost prevoda. **Metode.** Prevođenje i transkulturalno prilagođavanje upitnika IPS-*Anxiety* i IPS-*Avoidance* izvršeno je u skladu sa smernicama koje je dalo Međunarodno udruženje za farmakoekonomiju i praćenje ishoda lečenja (ISPOR). Ispitivanje pouzdanosti, faktorska analiza i validacija srpskog prevoda upitnika IPS-*Anxiety* i IPS-*Avoidance* je sprovedeno na uzorku od 485 studenata farmacije i medicine na Univerzitetu u Kragujevcu, Srbija. **Rezultati.** Srpski prevod upitnika IPS-*Anxiety* i IPS-*Avoidance* je pokazao visoku unutrašnju konzistenciju sa vrednostima Kronbahove alfe od 0,934 za IPS-*Anxiety,* odnosno 0,911 za IPS-*Avoidance.* Faktorska analiza IPS-*Anxiety* pokazala je da u okviru ove skale postoje dva faktora, koje smo nazvali Direktno iskustvo (9 pitanja) i Indirektno iskustvo (9 pitanja); faktorska analiza skale IPS-*Avoidance* takođe je ukazala na dva faktora koji se odnose na direktni i indirektni strah od injekcija. Studenti ženskog pola su imali veće vrednosti skora pokazujući i izraženiju fobiju od injekcija nego studenti muškog pola. Takođe, interesantno je da studenti farmacije ispoljavaju viši nivo fobije od injekcija nego studenti medicine, pri čemu je strah od injekcija više izražen

Correspondence to: Miloš N. Milosavljević, University of Kragujevac, Faculty of Medical Sciences, Svetozara Markovica 69, 34 000 Kragujevac, Serbia. E-mail: milosavljevicmilos91@gmail.com

kod studenata pete godine u odnosu na studente prve četiri godine studija. **Zaključak.** Srpski prevod upitnika IPS-*Anxiety* i IPS-*Avoidance* je pokazao dobre psihometrijske karakteristike na populaciji koju su činili studenti medicine i farmacije.

Ključne reči:
strah; injekcije; ankete i upitnici; prevođenje; srbija.

Introduction

The fear of injections is a type of anxiety disorder whose prevalence in the general population ranges from 3 to 5 percent [1]. Persons suffering from this kind of phobia feel excessive, irrational fear of needles, hospitals, surgery, wounds, pain, doctors or dentists [2]. The prevalence is highest in the youngest age, especially among girls [3–4], and is significantly higher among sick where by some estimates can reach up to 10% [5]. Patients with this type of anxiety experience serious mental and physical symptoms during invasive medical procedures, so they often avoid visiting a doctor and reject provision of medical services [2, 6]. This kind of behavior significantly reduces chances of cure [7] and has a negative impact on important social aspects of life, such as family planning, or choosing a profession [8].

There are several standardized and validated scales for assessment of this type of anxiety disorder [9]. Because of its simplicity and high reliability, the Injection Phobia Scale (IPS)-Anxiety and IPS-Avoidance are the most commonly used questionnaires [8]. It is the two-part questionnaire which consists of two separate scales, IPS-Anxiety and IPS-Avoidance. The first scale, IPS-Anxiety, has 18 questions, which are aimed to assess an intensity of fear among the respondents. The questions are actually the situations that can provoke fear among people with irrational fear of injections. A respondent assesses his/her fear in such situations by circling one of the answers on the Likert scale ranging from 0 to 4, where 0 indicates complete absence of fear and 4 indicates the highest intensity of fear. The second scale in this questionnaire, IPS-Avoidance, also has 18 questions, and in this part of the questionnaire the situations from the first part are used to assess how often respondents avoided medical procedures that are associated with fear of injections. There are three possible answers to each question on a scale from 0 to 2, where 0 means that respondent never avoids a specific medical procedure, while 2 means that he/she always avoids such a procedure. The result is shown as a total score, which is obtained by simply adding the values of answers on each individual issue, and it ranges from 0 to 72 for the first part of the questionnaire, and from 0 to 36 for the second part, with higher values indicating a higher intensity of fear [10].

A high reliability of this two-part questionnaire was demonstrated in the study [10], using a sample of 59 patients diagnosed with phobia of injections in 1992. The results of this validation study showed a high value of Cronbach's alpha for both subscales: 0.86 for IPS-Anxiety scale and 0.8 for IPS-Avoidance. In addition, there was a significant positive correlation between the two parts of the questionnaire ($r = 0.44$; $p < 0.05$). Content validity, criteria validity and structure validity of the questionnaire were confirmed in later methodologically well-placed research [11–13]. However, in order to use routinely this questionnaire in different speaking areas it is necessary to adapt the translation of the original version of the questionnaire to the cultural specificities and validate such adapted version.

The aim of this study was translating from English language to Serbian and cultural adaptation of the questionnaire IPS–Anxiety and IPS–Avoidance, with the assessment of reliability and validity of the translation.

Methods

Translation and cultural adaptation

Translation and cultural adaptation of the IPS–Anxiety and IPS–Avoidance to Serbian language was made according to the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) guidelines [14]. According to these recommendations, we first got permission from the author of the original scale, Professor Lars-Göran Öst, an expert in the field of cognitive behavioral therapy [15]. Then, the questionnaire was translated from English to Serbian by two independent translators, who were not part of the research team. The first translator was Mrs Jelena Jarčević, a graduated philologist in English language and literature and a member of the Association of Court and Technical Translators of Serbia. The second translator was Mrs. Dušica Lazić, a lecturer of English language at the Faculty of Medical Sciences, University of Kragujevac. At the meeting of the study investigators and the translators, two translations of the questionnaire were harmonized to one Serbian version. The harmonized Serbian version was back translated to English by Dr Zan Friščić, a native English speaker, citizen of Australia, who also participated in our study. Finally, at the second meeting of the investigators and the translators the final Serbian version of IPS-Anxiety and IPS–Avoidance questionnaire was agreed upon.

Clarity and comprehension of the final Serbian version of the questionnaire was tested in a pilot study. Ten students of pharmacy (at the Faculty of Medical Sciences, University of Kragujevac, Serbia) completed the questionnaire and we used feedback from them to made a few minor changes. The final Serbian version of IPS-Anxiety and IPS-Avoidance questionnaire was then prepared in the required number of copies and the reliability testing was conducted.

Study population and the sample size calculation

Our study population consisted of students of medicine and pharmacy at the Faculty of Medical Sciences, University of Kragujevac. The sample size was determined on the basis of the minimal power of the study 80%, on the maximal val-

ue of the first type error of 0.05 and by using the Pearson's correlation test. Since the correlations of responses to individual questions of the questionnaire and the total score of the original questionnaire were greater than 0.8 [8], we assumed the desired level of correlation in our study was 84%. Based on these parameters, minimal size of the study sample was 174 respondents. The reliability of the final Serbian version of the IPS-Anxiety and IPS-Avoidance questionnaire was tested on two occasions, on March the 14th, and on May the 14th, 2014. In total, 485 students voluntarily agreed to participate in the survey (375 of them were students of pharmacy and 110 were students of medicine) and the female : male ratio was 385 : 100. According to the year of the study, there were 86 students of the 2nd year, 174 students of the 3rd year, 175 students of the 4th year and 50 students of the 5th year of the study. The same study participants responded to the questionnaire on both occasions, although at the first time the investigators filled the questionnaires based on the interview with the participants, and after that the participants completed them by themselves.

The study was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac.

Reliability testing

Internal consistency of the Serbian version of the questionnaire was determined by calculating Cronbach's alpha for the whole questionnaire by the Spearman-Brown "prediction" formula [16].

After division of the IPS-Anxiety and IPS-Avoidance questionnaire into two separate scales, IPS-Anxiety and IPS-Avoidance, we calculated Cronbach's alpha for each subscale as well as the correlation matrices. Cronbach's alpha for the whole questionnaire was then calculated backward from the aplhas of the subscales [17]. The temporal stability of the questionnaire was tested by comparison of the internal consistency measures obtained by testing the same participants on two occasions, two months apart.

Validity

Content and construct validity of the Serbian translation of the IPS-Anxiety and IPS-Avoidance questionnaire were tested by the three-members panel of psychiatrists from the Clinic for psychiatry, Clinical Center Kragujevac. Criterion validaty of the Serbian translation of the IPS-Anxiety and IPS-Avoidance questionnaire was tested by comparison and correlation of its total score with the scores of the same study participants on the Injection Phobia Scale-Anxiety, Blood/Injection Fear Scale [18] and Medical Avoidance Survey [19].

Before factor analysis, we conducted the Barlett's test and the Kaiser-Meyer-Olkin (KMO) test. For the IPS-Anxiety, we used oblimin rotation with the Kaiser normalization and generalized least squares as a method of extraction. For the IPS-Avoidance, we used oblimin rotation with the Kaiser normalization and Principal component analysis as a method of extraction.

Results

Injection Phobia Scale (IPS)–Anxiety

The results are presented for 426 (87.83%) students due to the incomplete data. In the second round, the entire survey was completed by the same 425 students (87.01%). In both testing rounds, the female students scored higher on the scale showing more extensive injection phobia than the male students (Table 1). It is also interesting that the students of pharmacy had a higher level of injection phobia than the students of medicine, and those students of the fifth year of study felt more fear of injections than the students from the first four years. The mean results (with standard deviations) on the IPS-Anxiety according to the demographic parameters are shown in Table 1.

Table 1
Mean results ± SD on the Injection Phobia Scale (IPS)-Anxiety according to the demographic parameters in the first and in the second round

Demographic parameters	I round	II round
Gender (n)		
female		
I round: 343/426	10.24 ± 10.707	7.95 ± 10.198
II round: 338/425		
male		
I round: 83/426	7.02 ± 8.814	5.03 ± 10.568
II round: 87/425		
Medical sciences		
medicine	3.71 ± 4.294	1.73 ± 2.712
pharmacy	11.21 ± 11.009	8.91 ± 11.090
The year of the study		
the second	12.29 ± 12.970	8.92 ± 11.271
the third	7.00 ± 7.947	5.11 ± 7.805
the fourth	9.67 ± 10.689	7.90 ± 10.877
the fifth	13.00 ± 10.026	11.79 ± 13.599

SD – standard deviation.

The first round of tests

The first test showed high levels of internal consistency, with the Cronbach's alpha of 0.920 of the Serbian version of the IPS-Anxiety.

After dividing the IPS-Anxiety into two parts using the split-half method, the Cronbach's alphas were 0.863 and 0.832. The Spearman-Brown coefficient was 0.940 and the Guttman split-half coefficient was 0.920.

The second round of tests

The Cronbach's alpha of 0.934 indicated high levels of internal consistency of the Serbian version of the IPS-Anxiety.

After dividing the IPS-Anxiety into two parts using the split-half method, the Cronbach's alphas were 0.877 and 0.875. The Spearman-Brown coefficient was 0.940 and the Guttman split-half coefficient was 0.930.

There were no differences in the interclass correlation coefficient before and after division (Table 2).

Table 2

Inter-item correlation before and after dividing and interclass correlation coefficient before dividing for the Injection Phobia Scale (IPS)-Anxiety for the first and for the second round of tests

Time of testing	The first round of tests			The second round of tests		
Before dividing	Inter-item correlation	0.403 (0.792)		Inter-item correlation	0.459 (0,784)	
	Interclass correlation coefficient	Single measures	0.390 (0.356–0.427) $p = 0.000$	Interclass correlation coefficient	Single measures	0.438 (0.403–0.476) $p = 0.000$
		Average measures	0.920 (0.909–0.931) $p = 0.000$		Average measures	0.934 (0.924–0.942) $p = 0.000$
After dividing	Inter-item correlation	I	0.434 (0.497)	Inter-item correlation	I	0.464 (0.535)
		II	0.364 (0.699)		II	0.460 (0.668)

Factor analysis

The first round of tests

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO) was 0.919, and the Bartlett's test of sphericity: 4,840.893, $p < 0.05$.

The metod of extraction: Generalized least squares; The total explained variance of two-factor solution was 55.01%. The factor correlation matrix was 1–2: + 0.591; 1–3: -0.426; 2–3: -0.409.

Two extracted factors obtained by oblimin rotation with Kaiser normalization and generalized least squares as a method of extraction and eigenvalues for each factor and the amount of variance of the IPS-Anxiety explained by each factor are shown in Table 3. The matrix of weights of items and factors obtained with oblimin rotation with the Kaiser normalization of the two-factor solution for the IPS-Anxiety items are shown in Table 4.

Table 3

Extracted two factors obtained by oblimin rotation with the Kaiser normalization and generalized least squares as a method of extraction. Eigen values for each factor and amount of variance of (IPS)–Anxiety explained by each factor

Factor	Eigen value	Amount of variance explained (%)
Direct experience	7.817	43.428
Indirect experience	1.156	6.420

Table 4

Matrix of weights of the items and factors obtained with oblimin rotation with the Kaiser normalization of the two-factor solution for the Injection Phobia Scale (IPS)-Anxiety items

Items	Factor weights	
	DE	IE
When giving blood for analysis by taking a sample from your finger	0.158	**0.471**
When receiving an injection into the shoulder	0.101	**0.789**
When looking at a picture of a syringe and hypodermic needle	**0.585**	0.117
When feeling the smell of a hospital	**0.267**	0.167
When receiving an anesthetic injection at the dentists	0.173	**0.437**
When giving blood for analysis by obtaining a sample from your vein (venipuncture)	-0.143	**0.617**
When watching blood being drawn from another person's vein	**0.280**	0.048
When receiving an injection into the gluteus muscle	0.172	**0.651**
When watching a picture of a person receiving an injection	**0.922**	- 0.040
When listening to a conversation about injections	**0.651**	0.068
When looking at and touching the veins on the inside of the elbow	**0.228**	0.037
When watching a movie in which a person is receiving an injection	**0.676**	- 0.031
When watching a person getting an injection	**0.637**	0.053
When seeing a nurse in uniform	**0.198**	0.153
When getting your ears pierced	- 0.026	**0.547**
When receiving a vaccine injection	0.043	**0.828**
When having an intravenous injection	- 0.118	**0.683**
When watching a person have a blood sample drawn from his/her vein	**0.327**	0.032

IE – indirect experience; DE – direct experience.

Injection Phobia Scale (IPS) – Avoidance

The results are presented for 426 (87.42%) students due to the incomplete data. In the second round the entire survey was completed by 426 (87.83%). students The mean results (with standard deviations) on the IPS-Avoidance according to the demographic parameters are shown in Table 5.

Table 5

Mean results ± SD on the Injection Phobia Scale (IPS)-Avoidance according to the demographic parameters in the first and in the second round

Demographic parameters	I round	II round
Gender (n)		
female		
I round: 341/424	3.83 ± 4.813	4.60 ± 5.991
II round: 336/426		
male		
I round: 83/424	5.03 ± 5.469	3.04 ± 4.700
II round: 90/426		
Medical sciences		
medicine	1.94 ± 2.712	1.21 ± 1.887
pharmacy	5.56 ± 5.637	5.10 ± 6.175
The year of the study		
the second	6.05 ± 6.013	5.81 ± 7.163
the third	3.57 ± 4.453	3.11 ± 4.337
the fourth	4.90 ± 5.445	4.39 ± 5.848
the fifth	6.61 ± 5.935	5.44 ± 6.579

SD – standard deviation.

The first round of tests

The translated scale showed high levels of internal consistency, with the Cronbach's alpha of 0.920.

After dividing the IPS-Avoidance into two parts using the split-half method, the Cronbach's alphas were 0.765 and 0.778. The Spearman-Brown coefficient was 0.890 and the Guttman split-half coefficient was 0.885.

The second round of tests

The Cronbach's alpha of 0.911 indicated high levels of internal consistency of the Serbian version the IPS-Avoidance.

After dividing the IPS-Avoidance into two parts using the split-half method, the Cronbach's alphas were 0.847 and 0.834. The Spearman-Brown coefficient was 0.903 and the Guttman split-half coefficient was 0.894.

The Inter-item correlation before and after dividing the IPS-Avoidance scale for the first and for the second round of the tests are shown in Table 6.

Factor analysis

The first round of tests

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO): 0.879; the Bartlett's test of sphericity: 3282.275, $p < 0.05$.

The method of extraction: The principal component analysis; The total explained variance of the two-factor solution was 46.06%. The component correlation matrix: 1-2: + 0.413.

The extracted two factors obtained by oblimin rotation with the Kaiser normalization and eigenvalues for each factor and the amount of variance of the IPS–Avoidance explained by each factor are shown in Table 7.

The matrix of the factor weights of the items and factors with oblimin rotation with the Kaiser normalization of the two-factor solution for the IPS–Avoidance items are shown in Table 8.

Validity

Construct validity of the Serbian version of the IPS-Anxiety and IPS-Avoidance was analyzed and confirmed by the three-member panel of psychiatrists at the Clinic for Psychiatry, Clinical Center Kragujevac. As of the criterion validity, the total score of the IPS-Anxiety correlated significantly with the total scores of MAS (Spearman's correlation coefficient 0.315, $p < 0.001$) and BIFS (Spearman's correlation coefficient -0.332, $p < 0.001$). The total score of the IPS-Avoidance correlated significantly with the total scores of MAS (Spearman's correlation coefficient 0.364, $p < 0.001$) and BIFS (Spearman's correlation coefficient -0.332, $p < 0.001$). The one-trait-bi-method matrix is shown in the Table 9.

Table 6

Inter-item correlation before and after dividing the (IPS)-Avoidance scale for the first and for the second round of the tests

Time of testing	The first round of tests			The second round of tests		
Before dividing	Inter-item correlation	0.283 (0.819)		Inter-item correlation	0.370 (0.716)	
After dividing	Inter-item correlation	I	0.267 (0.567)	Inter-item correlation	I	0.385 (0.472)
		II	0.295 (0.646)		II	0.373 (0.677)

Table 7

Extracted two factors obtained by oblimin rotation with the Kaiser normalization. Eigenvalues for each factor and amount of variance of the Injection Phobia Scale (IPS)-Avoidance explained by each factor

Factor	Eigenvalue	Amount of variance explained (%)
Indirect experience	6.100	33.887
Direct experience	2.192	12.178

Table 8

Matrix of factor weights of the items and factors with oblimin rotation with the Kaiser normalization of the two-factor solution for Injection Phobia Scale (IPS)-Avoidance items

Items	Factor weights	
	IE	DE
When watching a picture of a person receiving an injection	**0.832**	-0.129
When watching a movie in which a person is receiving an injection	**0.777**	-0.106
When watching a person have a blood sample drawn from his/her vein	**0.765**	0.109
When watching a person getting an injection	**0.758**	0.143
When watching blood being drawn from another person's vein	**0.758**	0.143
When listening to a conversation about injections	**0.736**	-0.069
When looking at a picture of a syringe and hypodermic needle	**0.586**	-0.009
When looking at and touching the veins on the inside of the elbow	**0.404**	0.052
When seeing a nurse in uniform	**0.314**	0.131
When receiving an injection into the shoulder	-0.105	**0.827**
When receiving a vaccine injection	-0.004	**0.783**
When having an intravenous injection	0.112	**0.764**
When giving blood for analysis by obtaining a sample from your vein (venipuncture)	0.151	**0.721**
When receiving an injection into the gluteus muscle	0.058	**0.669**
When giving blood for analysis by taking a sample from your finger	0.040	**0.605**
When receiving an anesthetic injection at the dentists	-0.150	**0.544**
When getting your ears pierced	0.128	**0.332**
When feeling the smell of a hospital	0.151	**0.263**

IE – indirect experience; DE – direct experience.

Table 9

Spearman's correlation coefficients

	IPSA2	IPSAV2	BIFS2	MAS2	IPSA1	IPSAV1	BIFS1	MAS1
IPSA2	1							
IPSAV2	0.794*	1						
BIFS2	-0.332*	-0.339*	1					
MAS2	0.315*	0.364*	-0.425*	1				
IPSA1	0.786*	0.718*	- 0.282*	0.281*	1			
IPSAV1	0.660*	0.718*	-0.268*	0.289*	0.718*	1		
BIFS1	- 0.392*	-0.399*	0.597*	-0.518*	-0.407*	- 0.363*	1	
MAS1	0.181*	0.258*	-0.345*	0.662*	0.228*	0.282*	-0.477*	1

*** $p < 0.001$. IPSA2 – Injection Phobia Scale-Anxiety, filled in by the study participants themselves; IPSAV2 – Injection phobia scale (IPS) – Avoidance Scale, filled in by the study participants themselves; BIFS2 – Blood/Injection Fear Scale, filled in by the study participants themselves; MAS2 – Medical Avoidance Survey, filled in by the study participants themselves; IPSA1 – Injection Phobia Scale-Anxiety, filled in by the study investigators; IPSAV1 – Injection phobia scale (IPS)-Avoidance Scale, filled in by the study investigators; BIFS1 – Blood/Injection Fear Scale, filled in by the study investigators; MAS1 – Medical Avoidance Survey, filled in by the study investigators.**

Discussion

Both scales of the Professor Lars-Göran Öst's questionnaire [10] – IPS-Anxiety and IPS-Avoidance have excellent psychometric properties [11–13]. Reliability, contt validity, criteria validity and structure validity, as the most important psychometric properties were examined for the original version of the questionnaire in English. In accordance with the ISPOR guidelines we conducted the process of translating and cultural adapting of this questionnaire to Serbian language.

The psychometric properties of the Serbian version of the IPS-Anxiety and IPS-Avoidance scale are very similar with the original psychometric properties of these scales in English. The Serbian translation of these two scales showed high internal consistency, whereby the IPS-Anxiety scale showed slightly higher internal consistency than the IPS-Avoidance which is in accordance with the original scale [10]. Internal consistency of the translated questionnaire was higher when the participants answered to the questions by themselves, without interference of the investigator. Also, the Serbian version of the scale showed a satisfactory temporal stability.

Concerning the factor analysis, there were no significant differences about these two scales. Factor analysis of the first scale of this questionnaire – IPS-Anxiety suggests that there are two factors and this is in accordance with the results of other studies. We called these two factors as the Indirect Experience (refers to a type of fear which Olatunji et al. [12] called the Distal Fear) and the Direct Experience (refers to a type of fear which Olatunji et al. called the Contact Fear). The first factor analysis of the IPS-Anxiety scale was made in the study of Olatunji et al. [12] and these authors found 12 complex items and two lower order factors labeled as the Distal Fear and Contact Fear. However, the correlation between these two factors was high, so they considered that one-factor model would be quite good. The correlation between our two factors was also good and since the factors we extracted had no theoretical background, our conclusion about factor analysis of the IPS-Anxiety is in some way similar, i.e., the scale should not be divided into domains. On the other hand, the factor analysis of the IPS-Avoidance scale in English was not made previously, so we could not compare our results. Our factor analysis also suggested that there were two factors with a good correlation between them. These factors were similar to those in factor analysis for the IPS-Anxiety: nine questions referred to the indirect fear of injections (Indirect Experience), which people experience when they talk about injections or when they watch other people who receive injections, while the other nine questions referred to the direct fear of injections (Direct Experience), which people experience in the moments of receiving injections.

In both scales, the questions that had the highest average score where those that considered intravenous injection and injection of an anesthetic by dentist. Results from other studies also indicated high level of fear from these two kinds of injections [20–22]. Adherence to injections is important for a success of therapy and one of the most important factors which contribute to a low level of injection adherence is certainly phenomenon of injection phobia [23]. Considering these facts, it is very important to develop the instruments which could be used for the assessment of injection phobia. Not long ago, in Serbia, physicians have got the opportunity to use the Serbian version of the Medical Fear Survey (MFS), as an instrument for assessing Blood, Injury, Injections and Related Stimuli (BIIRS) phobia [24]. However, more precise assessment of injection anxiety and injection-related could be made by the Serbian translation of the IPS-Anxiety and IPS-Avoidance.

Conclusion

The Serbain version of the IPS-Anxiety and IPS-Avoidance showed similar psychometric properties and similar factorial structure with the original English version. However, we must confess that our study has potentially important limitations in terms of differences in sociodemographic characteristics of the study population compared to the general population in Serbia. Considering this fact, we believe it is necessary to test these translated Serbian versions of the IPS-Anxiety and IPS-Avoidance on more representative sample of the Serbian population before we can give a firm conclusion and recommendation about possibility of using these scales for measuring anxiety and avoidance of injections in Serbian socio-cultural milieu.

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Femoroacetabular impingement after the femoral neck fracture healed in a nonanatomical position

Femoroacetabularni impingement usled neanatomskog zarastanja kod preloma vrata butne kosti

Marko Mladenović*, Ivan Micić*†, Saša Milenković*†, Predrag Stojiljković*, Radoslav Barjaktarović‡

Clinical Center Niš, *Clinic for Orthopaedics Surgery, and Traumatology, Niš, Serbia; University of Niš, †Faculty of Medicine, Niš, Serbia; Military Medical Academy, ‡Clinic for Orthopaedic Surgery and Traumatology, Belgrade, Serbia

Abstract

Introduction. Femoroacetabular impingement is the result of the pathological conditions in the osseous acetabulum and/or the proximal femur. One of its causes is a non-anatomically healed femoral neck fracture. **Case report.** A male, aged 51, with a subcapital left femoral neck fracture was treated conservatively. The fracture healed 9 months later and although the patient was walking on crutches he suffered from pain in the left groin. The X-ray images showed the valgus and the retroposition of the left femoral head. The patient was operated on and intraoperatively the thickness and a bone prominence in the anterosuperior femoral neck area in the line of the previous fracture were found, which was pressing and spreading beneath the acetabular *labrum*, thus squeezing the acetabular cartilage. The *labrum* lesion which was found was the result of the mechanical pressure of the existing femoral neck deformity during the hip movements. Irretrievably damaged part of the *labrum* was resected and the anterosuperior femoral head-neck osteochondroplasty was done. One year after the surgery, the patient had no pain, he walked without limping, the impingement test was negative, the radiological parameters were adjusted and there were no signs of the avascular necrosis of the femoral head. **Conclusion.** The femoral neck fracture should be treated adequately with the full anatomical position and the proper internal fixation. If the deformity occurs as the result of a treatment, it should be removed as soon as possible to prevent the osteoarthritis of the hip.

Key words:
hip fractures; pain; femoroacetabular impingement; hip joint; orthopedic procedures; treatment outcome.

Apstrakt

Uvod. Femoroacetabularni poremećaj je rezultat patoloških stanja u acetabulumu i/ili proksimalnom femuru. Jedan od njegovih uzroka je i ne-anatomski zarastao prelom vrata femura. **Prikaz bolestnika.** Muškarac, star 51 godinu, sa subkapitalnim prelomom vrata leve butne kosti, lečen je konzervativno. Prelom je zarastao tek devet meseci kasnije. Pacijent je hodao na štakama i patio od bolova u predelu levog zgloba kuka. Rendgenske slike pokazale su valgus i retropoziciju leve femoralne glave. Pacijent je bio operisan i intraoperativno je nađeno zadebljanje u anterosuperiornom delu vrata butne kosti u nivou linije prethodne frakture. Ovo zadebljanje vršilo je pristisak i destrukciju acetabularne hrskavice i labruma. Lezija labruma koja je bila pronađena, bila je rezultat mehaničkog pritiska postojećeg deformiteta vratnog dela femura tokom kretanja kuka. Nepovratno oštećeni deo labrum-a je reseciran i urađena je osteohondroplastika anterosuperiorne femoralne glave i vrata. Godinu dana nakon operacije, pacijent je bio bez bolova, hodao je bez hramanja, a impingement test bio je negativan. Radiološki parametri bili su popravljeni i nije bilo znakova avaskularne nekroze femoralne glave. **Zaključak.** Frakturu vrata butne kosti treba adekvatno lečiti punom anatomskom repozicijom i pravilnom unutrašnjom fiksacijom. Ako se deformacija javlja kao rezultat lečenja, treba je ukloniti što je pre moguće kako bi se sprečio osteoartritis kuka.

Ključne reči:
kuk, prelomi; bol; femoroacetabularni sudar; kuk, zglob; ortopedske procedure; lečenje, ishod.

Introduction

Femoroacetabular impingement (FAI) is the cause of the hip pain in young adults and is a potential cause of a hip arthrosis development [1–9]. The minimal bone changes of the proximal femur and/or acetabulum combined with FAI as a pathophysiological mechanism, lead to an early *labrum* lesion and adjacent cartilage. FAI represents a marginal con-

Correspondence to: Marko Mladenović, Clinical Center of Niš, Clinic for Orthopedics Surgery and Traumatology, Bulevar dr Zorana Djindjić 48, 18 000 Niš, Serbia. E-mail: mladenovicdmarko@gmail.com

flict of the altered bone morphology between the rim of the acetabulum and the proximal femur of the hips [10, 11]. Flexion, internal rotation and the adduction of the hip provokes the unnatural contact between the femoral head-neck junction in the aspect of the asphericity with the anterosuperior upper rim of the acetabulum.

Two basic mechanisms of FAI have been described: cam impingement and pincer impingement, with a mixed type which combines the previous two. The cam impingement appears in younger, more often male adults and is the result of morphological, bone changes at the proximal femur, at the femoral head-neck junction in the form of a bone thickening or a cam, with the aspherical configuration of this junction. These changes, depending on the author who described it, were called "pistol grip" [12] or "tilt" deformity of the proximal femur [5]. Such changes can be seen in, slipped capital femoral epiphysis [13–15], Legg-Calve-Perthes disease [16, 17], avascular necrosis of the femoral head [18] and non-anatomically healed femoral neck fractures [19, 20].

During the flexion, the adduction and the internal rotation in the hip, anterosuperior, pathologically altered part of the femoral head- neck junction, spreads beneath the acetabular *labrum*, thus squeezing the *labrum* adjacent articular cartilage, while the *labrum* itself, initially, remains intact. Consequently separation of the acetabular cartilage from the *labrum* and subchondral bone occurs. Acetabular cartilage is irretrievably damaged, separating from the subchondral bone and the adjacent *labrum*, which leads to the degenerative changes of the hip [1, 21, 22]. Due to the fact that the *labrum* lesion occurs last, and the *labrum* is richly innervated, the groin pain intensifies in the developed stages of the disease appears [23] which is the reason why the cam FAI is malignant morfotype of the disease.

We presented a relatively rare form of a cam FAI, which is the result of a non-anatomically healed femoral neck fracture and also the results of the surgical treatment of the hip changes induced by trauma.

Case report

A male, aged 51, was hurt in July, 2009. Radiological finding was as follows: subcapital left femoral neck fracture with the dislocation of the femoral head in abduction. The patient was treated conservatively. The patient complained of a severe and constant pain in the left groin, the after conservative treatment was compleated, he could not walk without crutches and he was limping when walking. Nine months after the injury, physical examination of the patient confirmed limping on the left leg, slightly positive Trendelenburg's sign on the left leg and the limited motion in the hip joint: flexion up to 80°, internal rotation 20°, external rotation 45°, abduction is 40° and adduction up to 10°. The impingement test [1] was positive in the range of 40–80° of the flexion. The clinical findings were graded according to the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [24], and it was 76.

Radiological findings showed the healed subcapital femoral neck fracture without necrosis of the femoral head. The anteroposterior (AP) X-ray images of the hips (Figure 1) showed the value of collocapital diaphyseal angles (CCD) at the healthy right hip of 135° and at the injured, left hip 149° that was in favour of healing the femoral neck in abduction, i.e., *valgus* position of 19° [25]. Tonnis angle [26], had the value of 0° on both sides, the value of Wiberg angle [27], on the right hip was 43°, and on the injured, left hip, it was 38°. The value of the alpha angle, as the parameter of the femoral head convexity, as seen on the AP X-ray images of the hips, measured by the Nötzly method [28], on the right hip was 47°, and on the left hip was 57°. On the Dunn-Rippstein Müller lateral X-ray images, on the right hip, the alfa angle was 40° and on the left hip 73°, with the evident retroposition of the femoral head on the injured hip in relation to the healthy hip, with the disparity of 33° (Figure 2). The head of the femoral bone is not located centrally on the femoral neck, but it is located back and upwards. We measured the angle between the head of the femoral bone and its neck – the gama angle or collocapital angle [25]. Its normal value is -1° < gama < 2°; in our patient the gama angle was 5° (Figures 3 and 4).

The open surgical procedure, so-called trochanteric major flip osteotomy approach [29, 30], was performed in May 2010 under spinal anesthesia, 9 months after the hip injury. After the anterior "Z" capsulotomy, the controlled anterior surgical dislocation of the hip was performed.

Fig. 1 – Standardized anteroposterior radiographic image of the hips. Left: femoral bone neck fracture healed in abduction.

Fig. 2 – Standardized Dunn-Rippstein-Müller radiographic image of the hips. Left hip: femoral bone neck fracture healed in retroposition of the femoral head.

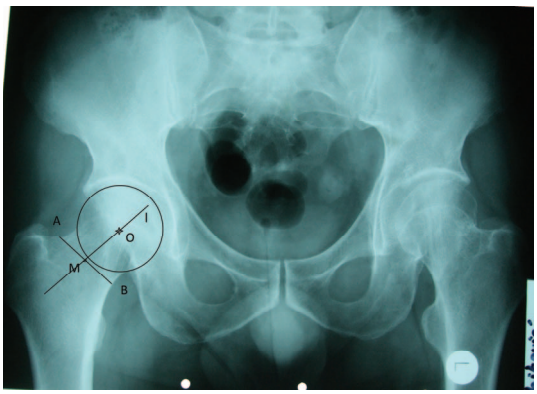

Fig. 3 – Femoral head axis (1) – AB, the line that connects upper and lower edge of the femoral bone neck on anteroposterior (AP) radiographic images of the hip, and front and rear edge of the neck on Dunn90°. M, the central point of the AB line. O, the center of femoral head rotation. OM is a line that represents the axis of femoral bone neck (axis 1) – it depends on the position of the femoral bone head center.

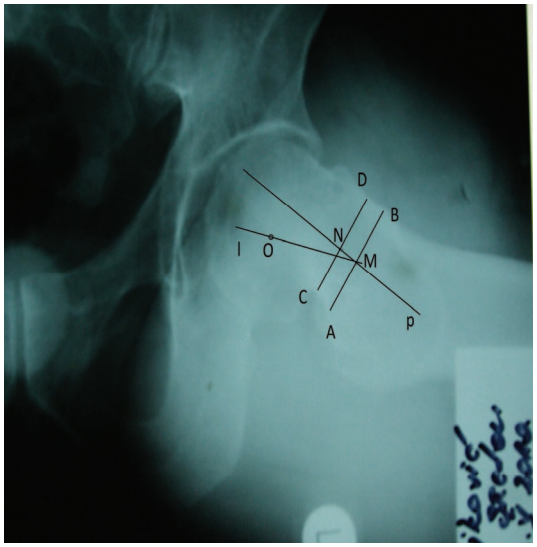

Fig. 4 – Femoral head axis represented by two parallel lines (p) – AB, the line drawn on the narrowest part of the femoral bone neck. CD, the line drawn 3–4 mm medially from the line AB. M, the central point of the AB line. N, the central point of the CD line. MN, the line that represents the axis of the medial third of the femoral bone neck, the position of which does not depend on the center of femoral head rotation. Axes l and p overlap (1 = p) when the head of the femur is located centrally on the neck. Gama angle is collo-capital angle of the femoral head created by l and p axes, the basic value in the pilot study of healthy hips is -1° < gama < 2°. In the case of our patient the angle gama is 5°.

Intraoperatively, in the anterosuperior femoral neck region, the femoral neck bone prominence in the shape of a cam was noticed (Figure 5). This prominence, i.e., cam, was osteotomized, taking into consideration that the size of the resection of the neck does not exceed 30% of its thickness [31–33] (Figure 6). A resection osteoplasty of the impinging site on the neck-haed junction was performed to improve femoroacetabular offset. In the upper anterosuperior part of the acetabulum, ranging from 12–17 hours [4, 34], the separation and serious damage of the *labrum* from its ledge was noticed. With no possibility of its refixation, the partial resection of the *labrum* was conducted. The reposition of the hip was performed, the joint capsule reconstructed and a major trochanter was fixed.

On the first postoperative day, active exercises in bed started and walking on the crutches was allowed from the second day with a touch down on the tip toes of the operated leg for 6 weeks after the surgery.

One year after the surgery, we found: the absence of the hip pain, walking without crutches and no limping, Trendelenburg sign negative, the impingement test also negative, the hip flexion was 90°, the internal rotation 15° and the other movements of the hip were within the normal range, and WOMAC score was 94.

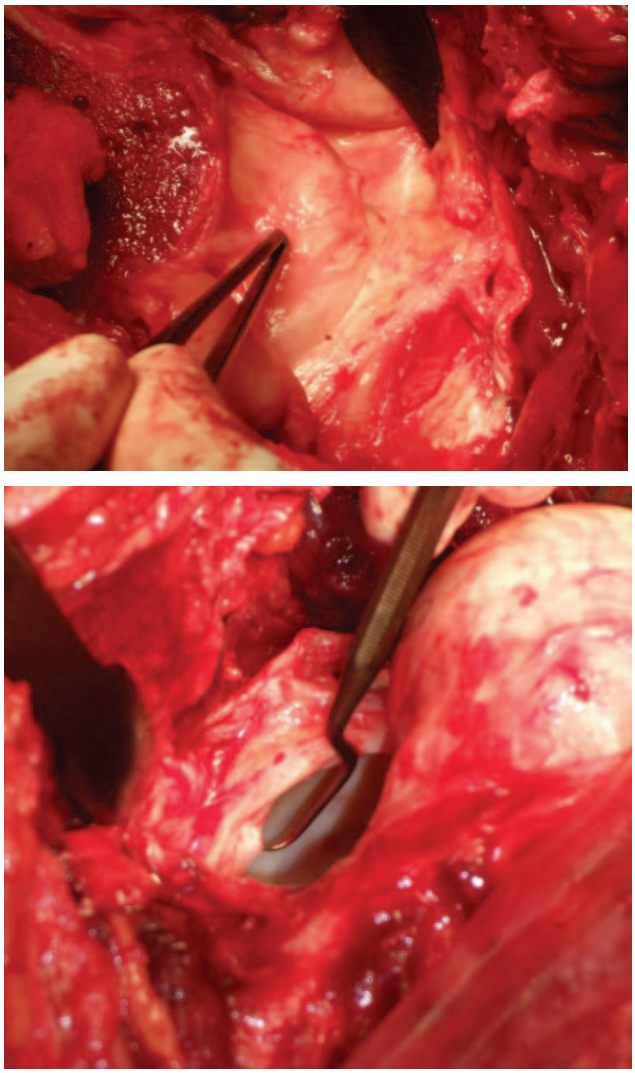

Fig. 5 – Intraoperative images: up – the cam deformity of femoral head and neck juncture impacts acetabular labrum; down – elevated and damaged acetabular labrum.

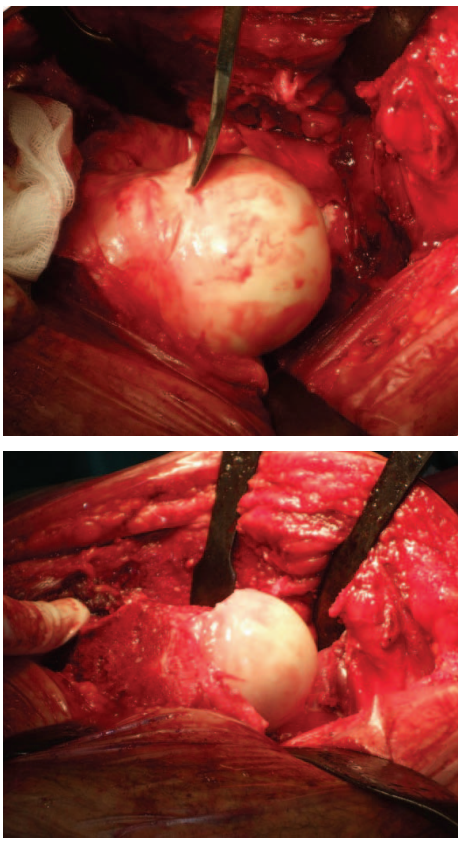

Fig. 6 – Intraoperative images: up – osteochondroplastica of the cam deformity of femoral head and neck juncture; down – the corrected cam deformity on the front side of head and neck of the femoral bone.

Postoperative Dunn-Rippstein-Müller profile X-rays images of the hip showed the reduction of the alfa angle values of the injured hip, from 73° to 50°, while on the AP X-rays the value of alpha angle was reduced from 57° to 52°, and the value of gama angle was reduced from 5° to 1° (Figure 7).Trochanter osteotomy of the femur,was healed, the femoral head was clearly contoured, without bone cysts and with no signs of the avascular necrosis development et heterotopic ossification.

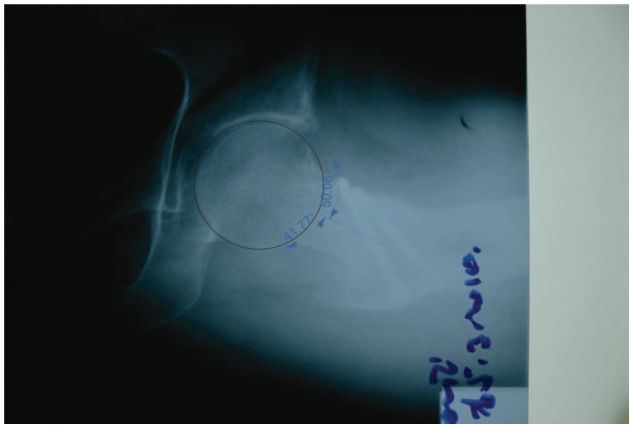

Fig. 7 – Postoperative Dunn-Rippstein-Müller radiographic image of the hip.

Discussion

There are few papers in the literature dealing with surgical correction of the deformity at the femoral head-neck junction after femoral neck fractures healed in a nonanatomical position [16, 17]. Thus Eijer et al. [19] in their paper, presented the treatment of 9 patients using the open surgical method, whereas all the other authors [3, 8, 13, 25, 27] presented in their papers the surgical treatment of the non-traumatic cam FAI form in patients with the pathological substrate at the femoral head-neck junction.

Dislocated fractures of the femoral neck, as a routine, are treated by the partial or the total hip replacement, and, in younger adults, the open reduction and internal fixation of the neck fracture are recommended. Only a few patients, with nondislocated femoral neck fractures are treated conservatively. In this presented case, dislocation of the femoral head in relation to the femoral neck was not noticed, and the treatment was conservatively completed. The patient's persistent symptoms and the subsequent clinical and radiographic examinations showed the presence of the bone prominence, i.e., cam in the anterosuperior region of the femoral neck in the area of the former fracture which led to the conclusion that it was very rare, trauma provoked form of the femoroacetabular impingement. The cam deformity is a consequence of osteogenesis on the place of femoral neck fracture. The dislocation of the femoral head caused an irritation and made place for excessive callus formation in the shape of the cam deformity, which damaged the cartilage and hip joint *labrum* with its presence, causing pain and limping. In this manner the femoral head kept the dislocated position.

In these malposition, the head-neck junction comes in contact with the acetabular rim of flexing the hip within normal range, especially when the leg rotated internally. The resulting sensation of pain may be caused by shear or compression of the acetabular *labrum*, which is known to carry proprioceptive and nociceptive nerve fibres similar to the knee meniscus [23].

Preoperative, subjective and objective symptoms of the patient were reduced and radiographic parameters showed, postoperatively that the values of the injured hip were brought to a level that approximated the values of the healthy hip. The reduction of alpha and gama angles to the normal values confirms the success of the surgical procedure, i.e., it confirms the sufficient extent of osteochondroplastica. Intraoperatively, these angles can be radiologically measured and adequately corrected, without relying on uncontrolled resection that can sometimes be insufficient. The values of these angles confirm the correct anatomical relation between femoral head and neck and the absence of the cam deformity, causing the femoral neck axes p and l to overlap. Thereby, the factors of pain and further hip joint *labrum* and cartilage damage are removed.

Using this procedure we created almost normal hip anatomy. Therefore, we believe that applied surgical technique and the approach to the treatment, are of the great importance in the treatment of the trauma-induced femoroacetabular impingement in symptomatic patients with the femoral neck fractures healed in a non-anatomical position.

Conclusion

The best prevention of this form of the impingement is based on the proper and immediate surgical treatment of the femoral neck fracture. Early surgical treatment of this form of the impingement is essential to prevent further degeneration and osteoarthritis of the hip.

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CASE REPORT

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Imaging features of bilateral vestibular neuritis

Radiološke karakteristike bilateralnog vestibularnog neuritisa

Duško Kozić*†, Slobodanka Lemajić-Komazec*‡, Mladen Bjelan*†, Jasmina Boban*†, Slavica Sotirović-Seničar§, Dejan Kostić‖¶

University of Novi Sad, *Faculty of Medicine, Novi Sad, Serbia; Oncology Institute of Vojvodina, †Diagnostic Imaging Centre, Sremska Kamenica, Novi Sad, Serbia; Clinical Center of Vojvodina, ‡Clinic of Otorhinolaryngology, §Center of Radiology, Novi Sad, Serbia; Military Medical Academy, ‖Institute of Radiology, Belgrade, Serbia; University of Defence, ¶Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Introduction. The magnetic resonance imaging (MRI) was found to be insensitive diagnostic modality in detecting the abnormalities in patients with vestibular neuritis. **Case report.** A 32-year-old man was admitted to hospital with clinical signs of acute vestibular neuritis. The conventional MRI was inconclusive, including 3 mm slice-thickness postcontrast study, while the postcontrast high resolution study with 1 mm slice-thikness, detected bilateral enhancement of the vestibulocochlear nerve's vestibular branch, consistent with inflammation. **Conclusion.** High-resolution 1 mm or submilimeter slices should be perfomed to evaluate patients with vestibular neuritis in order to increase the MRI sensitivity and improve correlation with clinical findings.

Key words:
vestibulocochlear nerve diseases; neuritis; bilateral vestibulopathy; diagnosis; magnetic resonance imaging.

Apstrakt

Uvod. Smatra se da magnetno-rezonantni imidžing (MRI) nije dovoljno osetljiva dijagnostička metoda za detekciju poremećaja kod bolesnika sa kliničkom slikom vestibularnog neuritisa. **Prikaz bolesnika.** Tridesetdvogodišnji muškarac je primljen u bolnicu sa kliničkom slikom akutnog vestibularnog neuritisa. Na snimcima konvencionalnog MRI pregleda, uključujući i postkontrastne snimke debljine 3 mm, nisu uočene patološke promene. Na postkontrastnim snimcima visoke rezolucije, debljine preseka 1 mm, uočeno je postkontrastno pojačanje intenziteta signala vestibularne grane oba vestibulokohlearna nerva, što je nalaz karakterističan za inflamaciju. **Zaključak.** Za evaluaciju bolesnika sa znacima vestibularnog neuritisa, a u cilju povećanja senzitivnosti MRI pregleda, neophodno je koristiti snimke visoke rezolucije, debljine preseka 1 mm ili submilimetarske preseke.

Ključne reči:
n. vestibulocochlearis, bolesti; neuritis; vestibulopatija, bilateralna; dijagnoza; magnetna rezonanca, snimanje.

Introduction

Vestibular neuritis (VN) still appears to be a controversial disease in clinical practice, with an unclear definition. According to Slivoniemi [1], the syndrome of VN is confined to the vestibular system with preserved hearing, while in labyrinthitis, reduced or distorted hearing in association with vertigo is evident. However, Murofushi et al. [2] also detected the involvement of the labyrinth in patients with clinical VN with or without inferior vestibular nerve involvement, using galvanic vestibular evoked myogenic potentials, supporting the hypothesis that VN could be caused by labyrinthine lesions. This observation suggested potential renaming of VN into "vestibular neurolabyrinthitis". Imaging findings, however, did not support the opinion of Murofushi et al. [2]. The data in the literature strongly suggested that the sensitivity of the magnetic resonance imaging (MRI) in the detection of VN was rather poor, both on the plain and contrast-enhanced MRI studies. The aim of this report is to prove the unequivocal role of the high-resolution MRI in detecting the involvement of vestibular system and labyrinth in patients with clinical VN.

Correspondence to: Mladen Bjelan, Oncology Institute of Vojvodina, Diagnostic Imaging Center, Put Dr. Goldmana 4, 21 204 Sremska Kamenica, Serbia. E-mail: mladen.bjelan@gmail.com

Case report

A 32-year-old man was admitted to the hospital with clinical signs of acute rotatory vertigo, nausea, and vomiting. There was no hearing loss, tinnitus or other hearing disturbances. The cardiopulmonary examination detected no abnormalities. The clinical otoneurologic examination revealed the presence of horizontal rotatory nystagmus. The Dix-Hallpike test for benign paroxysmal positional vertigo was negative. The video head impulse test of the lateral semicircular canal found the gain of the vestibulocochlear reflex of 0.46 on the right and 0.89 on the left. The computed tomography of the brain and skull base revealed no abnormalities. Betahistine dihydrochloride in a dosage of 24 mg/7 days was prescribed. On the control examination, the gain of vestibulocochlear reflex was 0.55 on the right and 0.89 on the left. The viral tests were negative on herpes simplex virus, type 1 (IgM 0.14 arbU/mL – normal range if less than 1.2 arbU/mL, IgG 2.0 – normal range if less than 5 arbU/mL). IgM on Ebstein-Barr virus was 0.5 arbU/mL (normal range if < 0.8 arbU/mL), while IgG was positive – 2.91 arbU/mL (normal range if < 0.8 arbU/mL). IgM on adenovirus was 0.3 arbU/mL (normal range if < 0.8 arbU/mL), while IgG was slightly positive – 1.41 arbU/mL (normal range if < 1.1 arbU/mL). No improvement was evident on the follow-up otologic examination and the MRI scan of temporal bones was ordered. The conventional head MRI protocol included 5 mm slice thickness T1-weighted (T1W), T2-weighted (T2W), fluid attenuated inversion recovery (FLAIR) images in the axial plane and coronal T2W images, as well as the diffusion-weighted imaging in the axial plane (slice thickness of 5 mm, TR 4900 ms, TE 111 ms, b0, 500, 1000 mm^2/s). For the detailed evaluation of inner ear structures the MRI protocol was complemented with constructive interference in steady-state – 3D constructive interference in steady state (3D CISS) (slice thickness 0.7 mm, TR 8.4 ms, TE 3.4 ms), axial T1W fat sat (slice thickness 3 mm, TR 700 ms, TE 8.9 ms) and contrast-enhanced T1W fat sat sequence in axial and coronal planes as well as sagittal three-dimensional T1-weighted gradient echo, magnetization-prepared rapid gradient echo (MPRAGE) sequence (slice thickness 1 mm, TR 1450 ms, TE 4.7 ms). MPRAGE sequence clearly detected marked contrast enhancement of the vestibular branch of the vestibulocochlear nerves bilaterally (Figure 1).

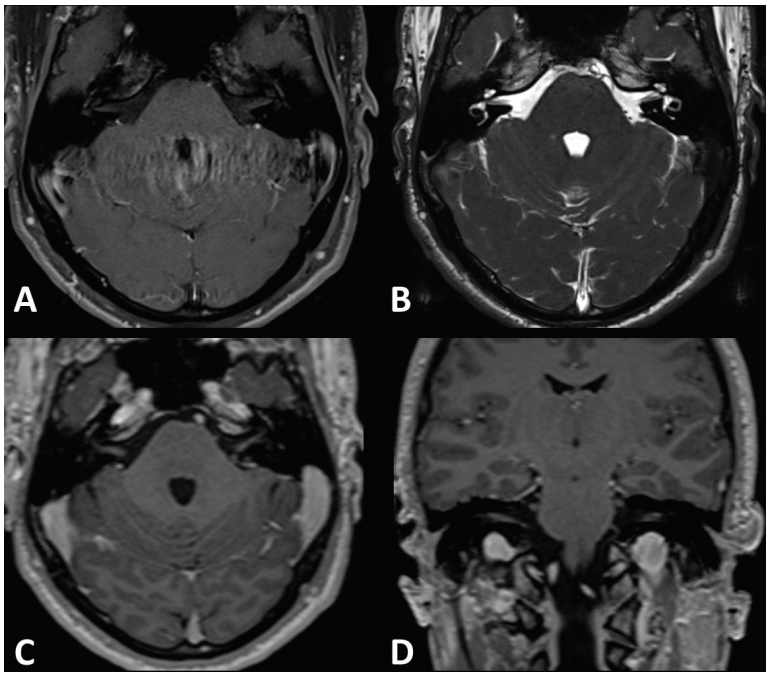

Fig. 1 – A) No abnormalities evident on conventional, 3 mm slice thickness postcontrast T1W fat sat sequence; B) 3D CISS, 0.7 mm slice thickness; C) Marked contrast enhancement of the vestibulocochlear nerves bilaterally on postcontrast 1 mm slice thickness magnetization-prepared rapid gradient echo (MPRAGE) sequence in axial, and D) coronal plane.

Discussion

In patients with VN, the MRI is usually ordered to exclude other neurologic disorders, since it was found to be insensitive to confirming the presence of the disease itself. Strupp et al. [3] did not detect contrast enhancement of the labyrinth, vestibulocochlear nerve or vestibular ganglion, in any of the 60 patients with acute idiopathic VN (confirmed by clinical examination and caloric irrigation), even when high doses of gadolinium (0.2 mmoL/kg) were administered. In that study, the high-resolution MRI was performed between days 3 and 30 after symptoms' onset. Hasuike at al. [4] also reported no MRI abnormalities in all 8 patients with VN. The only report in the literature, showing that the postcontrast MRI might be useful in detection of the vestibular nerve enhancement in patients with VN, was published by Karlberg et al. [5]. This study was performed on 3T scanner, with a slice thickness of 2 mm. We also found the postcontrast images

with a slice thickness of 3 mm inconclusive in our patient, but 1 mm slice thickness MPRAGE sequence detected bilateral involvement of the vestibulocochlear nerves. Fundakowski et al. [6] found decreased size both in the vestibular nerve cross-sectional area and height in patients with VN measured on parasagittal 3D CISS MRI. Recently, the profiles of afferent dysfunction in a cross-section of patients with acute VN was characterized, using the tests of otolith and semicircular canal function, sensitive to each of the five vestibular end organs. Acute vestibular neuritis most often affects both vestibular nerve divisions. The horizontal canal-plane video head impulse tests alone identified the superior nerve dysfunction in all patients with vestibular neuritis tested acutely, whereas both cervical/vestibular evoked myogenic potentials and the posterior canal-plane video head impulse tests were necessary for diagnosing the inferior vestibular nerve involvement [7]. Another unusual finding in our patient was bilateral involvement, very atypical for classical unilateral VN. Bilateral involvement could be associated with the autoimmune processes, like rheumatoid arthritis, Cogan's syndrome, polychondritis or lupus erythematosus. In our patient, no acute infection could have been associated with acute vestibulopathy, while IgG on Ebstein-Barr virus was markedly positive. Several studies on immune-mediated sensorineural hearing loss detected IgG antibodies against the membranous labyrinth. The pathogenicity of these antibodies remained unclear, however, their appearance seemed to suggest organ-specific immune dysregulation [8–11]. Nevertheless, the diagnosis of autoimmune inner ear disorders is still problematic due to a lack of universally accepted set of the diagnostic criteria or diagnostic test.

Conclusion

This report challenges the reevaluation of the current approach to the high-resolution MRI role in the evaluation of patients with VN. This appears to be the second report suggesting that < 3 mm thin slices do increase the sensitivity of detection of the vestibular nerves involvement on the postcontrast MRI studies. Bilateral involvement documented by the high-resolution MRI supports the consideration of VN as an immune-related vestibular neuropathy in certain circumstances.

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CASE REPORT

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Melena as a first sign of metastatic hepatic angiosarcoma: A case report

Melena kao prvi znak metastatskog angiosarkoma jetre

Aleksandra Sokić-Milutinović*†, Ljubiša Tončev†, Tijana Glišić*†, Vera Matović‡, Marjan Micev§, Srdjan Djuranović*†, Miodrag Krstić*†

University of Belgrade, *Faculty of Medicine, Belgrade, Serbia; Clinical Centre of Serbia, †Clinic for Gastroenterology and Hepatology, ‡Emergency Center, §Department of Pathology, Belgrade, Serbia

Abstract

Introduction. Angiosarcomas are malignant tumors of vascular endothelium that may arise from different locations. Although primary hepatic angiosarcoma accounts for only 1.8% of primary liver tumors, it is the most common malignant mesenchymal tumor of the liver. We report a case of primary hepatic angiosarcoma with melena as an unusual initial manifestation of this extremely rare tumor. **Case report.** Forty-four-years old patient with melena was referred to our Clinic because melena was not resolved after repeated argon plasma coagulation of bleeding lesions during esophagogastroduodenoscopy in the regional hospital. Abdominal ultrasound and multislice computed tomography (MSCT) revealed enlarged liver, with focal lesion 6 cm in diameter localized in the left lobe with multiple satellite lesions in both liver lobes, enlarged spleen and extremely dilated and long umbilical vein. Double-balloon enteroscopy and video capsule endoscopy detected the multiple bleeding vascular lesions in the small bowel. Histopathological examination and immunohistochemistry of the small bowel lesions revealed malignant mesenchymal proliferation with vascular/endothelium differentiation of neoplastic cells. The patient was diagnosed with metastatic angiosarcoma probably of hepatic origin with metastasis in the small bowel, that caused melena, and in the lumbar spine, causing back pain. **Conclusion.** Rare causes of melena include bleeding from primary or metastatic hemangiosarcoma localized in the gastrointestinal tract, especially small bowel.

Key words:
hemangiosarcoma; liver neoplasms; melena; diagnostic techniques and procedures; diagnosis, differential; palliative care.

Apstrakt

Uvod. Angiosarkomi su maligni tumori vaskularnog endotela koji mogu nastati na različitim lokacijama. Iako primarni angiosarkomi jetre čine samo 1.8% primarnih tumora jetre to su najčešći maligni mezenhimalni tumori jetre. Prikazujemo slučaj primarnog angiosarkoma jetre sa melenom kao neobičnom inicijalnom manifestacijom ovog vrlo retkog tumora. **Prikaz slučaja.** Bolesnik star 44 godine upućen je na našu Kliniku zbog melene koja je perzistirala nakon ponavljanih argon plazma koagulacija krvarećih lezija tokom ezofagogastroduodenoskopije u regionalnoj bolnici. Ultrazvuk i multislajsna kompjuterska tomografija (MSCT) abdomena ukazali su na uvećanu jetru sa fokalnom lezijom levog lobusa promera 6 cm i multiplim satelitskim lezijama u oba lobusa, uvećanu slezinu, kao i ekstremno proširenu i dugačku umbilikalnu venu. *Double balloon* enteroskopijom i endoskopskom video kapsulom uočene su brojne krvareće vaskularne lezije u tankom crevu. Patohistološki pregled i imunohistohemijska bojenja biopsija lezija iz tankog creva ukazala su na maligni mezenhimski tumor vaskularnog/endotelnog porekla. Zaključeno je da se kod bolesnika radi o metastatkom angiosarkomu sa najverovatnijim primarnim ishodištem u jetri i metastazama u tankom crevu, što je uzrokovalo melenu, i kičmenom stubu, što je uzrokovalo bol u leđima. **Zaključak.** Retki uzroci melene uključuju krvarenje iz primarnog ili metastatskog hemangiosarkoma lokalizovanog u gastrointestinalnom traktu, posebno u tankom crevu.

Ključne reči:
hemangiosarkom; jetra, neoplazme; melena; dijagnostičke tehnike i procedure; dijagnoza, diferencijalna; lečenje, palijativno.

Correspondence to: Aleksandra Sokic-Milutinovic, Clinical Center of Serbia, Clinic for Gastroenterology and Hepatology, Koste Todorovica 6, 11 000 Belgrade, Serbia. Email: asokicmilutinovic@gmail.com

Introduction

Angiosarcomas are malignant tumors of vascular endothelium that may arise from different locations. They occur most commonly in the skin and soft tissue. Although primary hepatic angiosarcoma accounts for only 2% of primary liver tumors, it is the most common malignant mesenchymal tumor of the liver in adults [1]. Early reports of hepatic angiosarcoma focused on its association with environmental chemical carcinogens, such as vinyl chloride, thorium dioxide (Thorotrast) and arsenic, but exposure to these agents is now rare. Other known risk factors include use of androgenic steroids, oral contraceptives and cyclophosphamide, but most of these tumors nowadays occur in the absence of known risk factors [1].

We report a case of metastatic hepatic angiosarcoma with melena as first and unusual manifestation of this exceedingly rare tumor.

Case report

A 44-year-old man, a professional truck driver, was referred to the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, with persistent melena. Prior to admission to our Clinic, the patient was admitted and treated for one month in the regional hospital where esophagogastroduodenoscopy (EGD) revealed two duodenal bleeding lesions treated with several argon plasma coagulation (APC) sessions. After eight APC sessions in a regional hospital, melena persisted and patient was referred to our Clinic. On the day of admission, patient complained of exhaustion, nausea and melena and reported lower back pain he suffered from for past 4 years. The patient`s past medical history was not significant, except for spine injury in a traffic accident 4 years ago and subsequent operation with osteosynthetic metal implant in lumbosacral spine. He denied exposure to environmental toxins. Physical examination revealed enlarged liver 2 cm below right costal margin in the medioclavicular line and enlarged spleen palpable for 1cm below left costal margin in the medioclavicular line. Initial laboratory findings were haemoglobin (Hb) 10.2 g/dL, haematocrit (Hct) 30%, platelet count (Plt) 76 x 10^9/L, prothrombin time (PT) 15.8 s, albumin 29 g/L, alkaline phosphatase (ALP) 186 IU/L, aspartate transaminase (AST) 40 IU/L, alanine transaminase (ALT) 45 IU, erythrocyte sedimentation rate (ESR) 8 mm/h. Since the patient presented with hepatosplenomegaly of unknown origin immediately upon admission, immunology profiles and serology for viral hepatitis were ordered. Hepatitis A, B and C and immunology profiles [antinuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), antimitochondrial antibody (AMA), perinuclear antineutrophil cytoplasmatic antibodies (pANCA)] were negative. Further investigation, after ultrasound, revealed focal lesions of the liver, and all tested tumor markers including: alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carcinogen 19-9 (CA 19-9) and 125 (CA 125), total prostatic specific antigen (tPSA), free prostatic specific antigen (fPSA), beta human chorionic gonadotropin (βHCG), were within normal range. The chest radiography was normal. Ultrasound of the abdomen showed enlarged liver with a large 6-cm mass in the left liver lobe (Figure 1), multiple up to 2 cm small focal lesions in both lobes of the liver, extremely dilated (20 mm in diameter) and long umbilical vein with turbulent flow, enlarged spleen, but no ascites. EGD was performed upon admission and revealed three mucosal non-bleeding lesions resembling stigmata of previous APC in second duodenal portion. Colonoscopy and barium follow-through were normal. Multislice computed tomography (MSCT) angiography of the abdomen identified in the segment IV and VII of the liver two large focal lesions with the CT characteristics resembling cavernous haemangioma (Figure 2) together with numerous intraabdominal collaterals but no thrombosis of *v. portae*, *v. lienalis* or *v. mesenterica* that would explain recanalised umbilical vein.

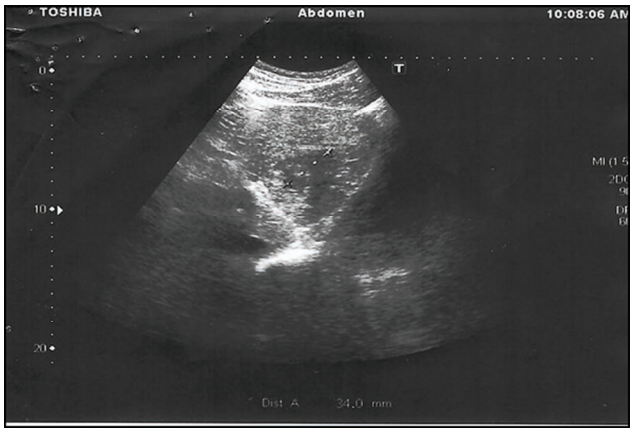


Fig. 1 – Large mass in left lobe of the liver.

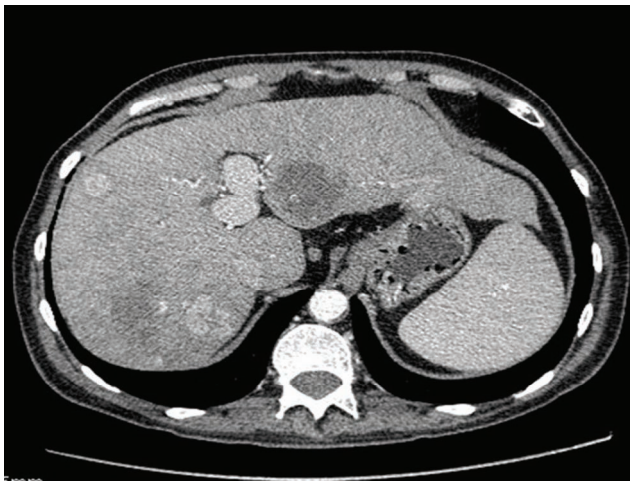


Fig. 2 – Multislice computed tomography shows two large focal lesions in the IV and VII segment of the liver.

Peroral double balloon enteroscopy was performed and revealed few larger non-bleeding pseudopolypoid lesions with hyperemic edge and central umbilication that were considered to be of a vascular origin, thus contraindicated for biopsy. The patient was treated with sandostatin and beta-blockers that reduced frequency of melena and need for blood transfusion. Due to the metal implant in this patient, we could not order magnetic resonance imaging (MRI), therefore liver scintigraphy and blood pool were performed

in order to clarify characteristics of the liver lesions and these results were suggesting the vascular lesions in both liver lobes. Since melena occurred again, M2A video capsule was performed and showed hyperemic zone without bleeding in duodenum (Figure 3a), active bleeding in distal duodenum without visible lesion (Figure 3b), one prominent bleeding lesion in distal jejunum that could correspond to varix or tumor (Figure 3c) and another large lesion in proximal ileum (Figure 3d). Percutaneous liver biopsy was not performed because of the high bleeding risk due to the vascularity of the lesions and patient's impaired coagulation. The patient was discharged in a stable condition with the diagnosis of probable liver cavernous hemangioma and enteropathy due to portal hypertension with advised supportive therapy including proton pump inhibitors (PPI), Lanreotide and β-blockers. One month after discharge, the patient was readmitted to our hospital with persistent melena, back pain and weight loss. Laboratory data at readmission were: Hb 64g/L, Hct 19%, platelet 66×10^9, albumin 25 g/L, alkaline phosphatase 250 IU, D-dimer > 4000 mg/L, vWF (von Willebrand factor) > 150%. Upon readmission, the abdominal ultrasound and MSCT scan showed enlargement of previously diagnosed liver focal lesions and massive osteolytic defects in the lumbal spine, and therefore, the initial diagnosis was challenged. EGD was repeated and showed the focal pseudopolypoid lesions with umbilication and scattered bleeding, especially in third portion of duodenum. At this point it was decided that biopsies from the small bowel lesions are lower risk than liver biopsy. The histological examination of the small bowel lesions revealed malignant mesenchymal proliferation with vascular/endothelium differentiation of neoplastic cells. The tumor cells stained positive for vimentin and CD31.

In the light of histological appearance (Figure 4a), positive immunohistochemical staining for CD 31 (Figure 4b) and evident progression of the disease, we concluded that patient suffered from primary hepatic angiosarcoma with metastatic lesions affecting small bowel that caused melena and lumbal spine that caused back pain. The patient was referred to his regional Oncology Center where he was given only symptomatic treatment due to deteriorated general condition. The patient died two months after the diagnosis was made.

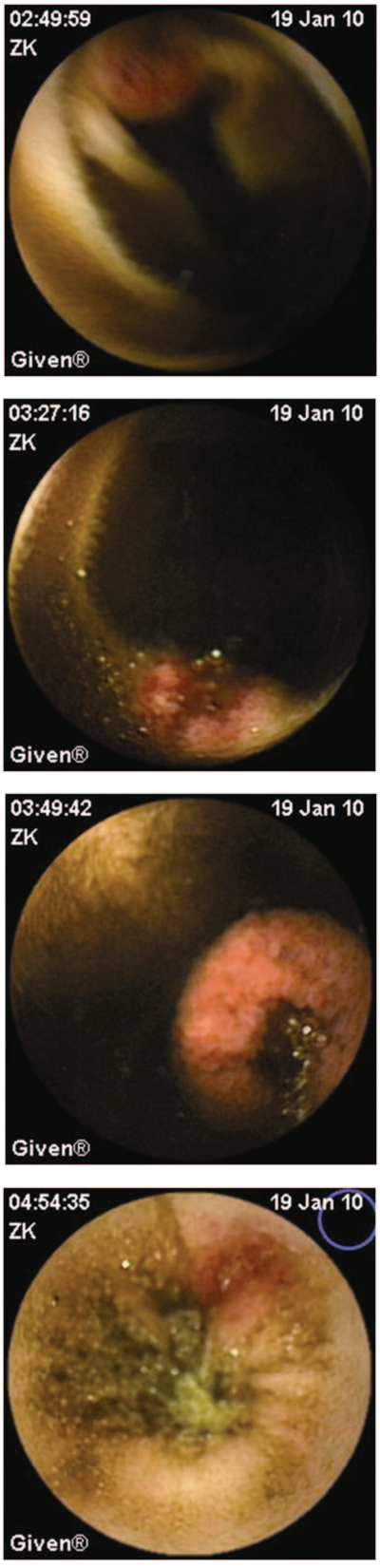
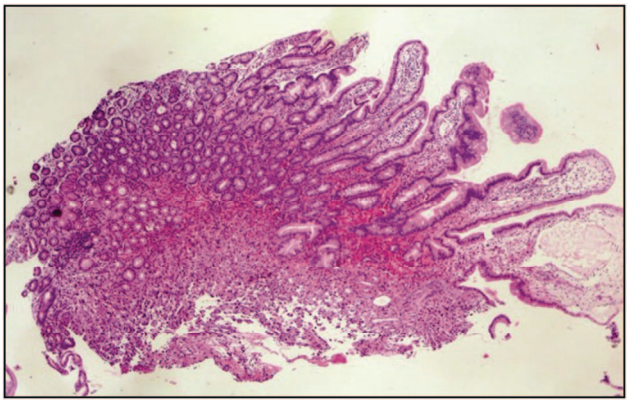


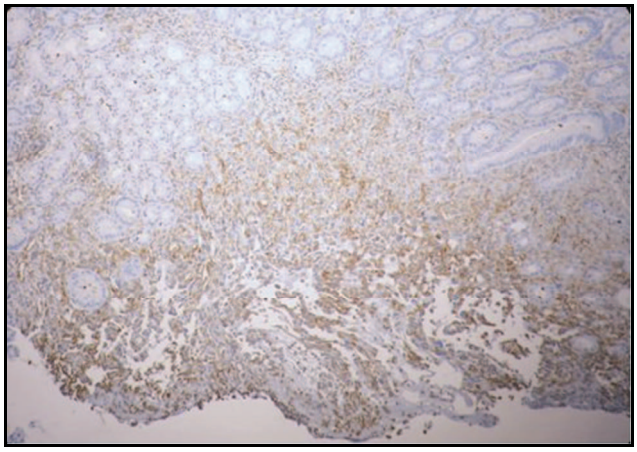
Fig. 3 – M2A video capsule shows: a) Hyperemic zone without bleeding in duodenum; b) Fresh bleeding in distal duodenum without visible lesion; c) Bleeding lesion in distal jejunum; d) Metastatic lesion in proximal ileum.

Discussion

Angiosarcomas are rare malignant tumors arising from vascular endothelium that account for less than 1% of all soft tissue sarcomas. The development of the tumor is in 25% of all cases related to previous exposure to environmental toxins such as thorium dioxide (Thorotrast), polyvinyl chloride monomers and arsenic-containing insecticides, while in the majority of patients no underlying risk factor is identified [1]. Primary hepatic angiosarcoma shows a male predominance of 3 : 1 and majority of patients are diagnosed between the ages of 50 and 59 [1, 2].



a)



b)

Fig. 4 – Histopathological findings: a) Hematoxylin and eosin staining of the small bowel lesion; b) Atypical endothelial cells with CD31 positive staining. CD 31 is considered the most reliable immunohistochemical endothelial cell marker suggesting vascular origin of the lesions in the small bowel.

Clinical presentation of hepatic angiosarcoma usually includes fatigue, weight loss, ascites, jaundice, abdominal pain in the right upper quadrant and sometimes fever [1, 3, 4], but acute liver failure and acute intra-abdominal hemorrhage from primary tumor rupture as initial presentation of hepatic angiosarcoma were also described. According to the data from the relevant literature, acute abdominal bleeding occurs spontaneously or after instrumentation in 17% to 27% of hepatic angiosarcoma patients [1].

Although majority of patients with angiosarcoma have metastatic lesions at the time of presentation, only 9% of patients present with symptoms from distant metastasis as in the case of our patient [1]. The most common site of metastasis is the lung, followed by the spleen [1].

Angiosarcoma occurs very rarely in the intestinal tract as either primary or metastatic malignancy. In a case series published by Allison et al. [5], 8 cases of angiosarcoma involving gastrointestinal tract were described and out of 5 patients whose initial presentation were signs of gastrointestinal bleeding, 2 had melena with primary lesion localized in duodenum in one and in jejunum in the other patient. Another case series by Taxy and Battifora [6] described 3 patients with angiosarcomas involving small intestine and one of these had signs of gastrointestinal bleeding. Ni et al. [7] described primary angiosarcoma of the small intestine with metastasis to the liver. This patient had single lesion localized in the jejunum and multiple liver lesions, and in this case, there was no gastrointestinal bleeding. Small intestine bleeding from multifocal jejunal angiosarcoma that presented with signs of anemia and melena was also described [8].

Previously published sporadic case reports indicate that small bowel is a rare site of metastatic localization of hepatic angiosarcoma [9, 10] originating from subcutaneous angiosarcoma of the head [11], aortic endothelioma [12–14], thyroid gland [15] and spleen [16]. Multifocal epitheloid angiosarcoma of the small intestine originating from the skin in one and of unclear origin in the other patient was also described [17, 18].

Metastasis of hepatic angiosarcoma to the gastric vein [19] and to the gastric mucosa without bleeding from the lesion was previously reported [20].

In our patient, the diagnosis of metastatic hepatic angiosarcoma that affected lumbal spine, duodenum and small bowel was supported by the fact that a large liver lesion with satellites was major finding and the lesions in the small bowel were multicentric. Extensive diagnostic workup in our patient did not reveal any other possible primary localization of angiosarcoma.

Forbes et al. [3] reviewed data from 25 adult patients diagnosed with hepatic sarcoma (8 with angiosarcoma). Since differentiation between the primary and the secondary hepatic sarcoma is difficult in clinical setting, the authors suggested histological appearance an important guide and that angiosarcomas and undifferentiated sarcomas almost certainly originated from liver, which would support the idea that, in our patient, the primary lesion was localized in the liver. Recent data suggests that immunohistochemical positivity for endothelial markers such as CD34 and CD31 can confirm vascular nature of the tumor. CD31 is considered as the most reliable marker [1]. Erythroblast transformation specific related gene (ERG) is another sensitive marker of endothelial differentiation. According to Sullivan et al. [21] ERG and CD31 have higher sensitivity than CD34 and are valuable for the cytological diagnosis of angiosarcoma.

Prognosis in patients with hepatic angiosarcoma is very poor due to the fact that in majority of cases diagnosis is made in the metastatic stadium of the disease. Long-term survival has been described after complete surgical removal of isolated hepatic angiosarcoma [22]. Since angiosarcoma are resistant to chemotherapy and radiotherapy majority of patients die within 6 months of diagnosis, while approximately 3% of patients live more than 2 years. Survival after liver transplant is less than 7 months due to high recurrence rates [1].

Conclusion

Small intestine is rarely the site of primary or metastatic angiosarcoma, with no specific symptoms that would facilitate clinical diagnosis, but involvement of the gastrointestinal tract should be suspected in patients presenting with signs of gastrointestinal bleeding and the focal vascular liver lesions.

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I N M E M O R I A M

prof. dr sc. med. VLADETA MARINKOVIĆ
pukovnik u penziji
(1936–2018)

Pukovnik prof. dr sc. med. Vladeta Marinković je rođen 18.03.1936. godine u Bruvnu (Lika) u učiteljskoj porodici. Osmogodišnju školu završio je u Banatskom Karađorđevu, a gimnaziju u Zrenjaninu sa odličnim uspehom. Diplomu Medicinskog fakulteta Univerziteta u Beogradu stekao je 1962. godine. Tokom studija radio je u Odboru za zdravstveno prosvećivanje na Fakultetu i Sekciji za društveno-politički rad. Kao student učestvovao je četiri puta na saveznim radnim akcijama, a dva puta bio udarnik i komandir čete.

Po završenom lekarskom stažu i odsluženju vojnog roka unapređen je u čin poručnika. Početkom 1964. godine postavljen je za referenta sanitetske službe i upravnika ambulante Školskog centra veze u Ljubljani. Na toj dužnosti ostao je do 1970. godine, kada je upućen na specijalizaciju iz infektologije u Vojnomedicinsku akademiju (VMA) u Beogradu, gde je 1973. godine položio specijalistički ispit iz infektologije sa odličnim uspehom. Kao specijalista odmah je počeo da radi u Opštem odeljenju, potom u Odeljenju za crevne zarazne bolesti i ambulanti Klinike za zarazne bolesti VMA. Po prelasku u novu zgradu VMA radio je četiri godine na I Odeljenju Klinike, potom je postavljen za načelnika ambulante Klinike, pa načelnika I Odeljenja Klinike za zarazne bolesti VMA. Načelnik Klinike za infektivne i tropske bolesti VMA bio je od 1994. do 1996. godine.

Doktor Marinković izabran je za asistenta iz predmeta Infektologija 1981. godine. Doktorsku disertaciju pod nazivom *„Prilog dijagnostici funkcionalne hiperbilirubinemije u oceni sposobnosti za vojnu službu“* odbranio je 14. marta 1989. godine, kao potpukovnik i načelnik ambulante Klinike za zarazne bolesti. Do tada je već bio autor ili koautor u 42 rada (u 14 prvi autor, u 28 koautor). Kako je ispunjavao sve uslove, asistent dr Marinković je 1989. godine izabran u zvanje docenta, a na 365. sednici NNV VMA održanoj 19. maja 1995. izabran je za vanrednog profesora.

Radeći kao lekar na specijalizaciji, a kasnije kao specijalista infektolog u Klinici za zarazne bolesti VMA, dr Marinković je ispoljavao veliku motivaciju i želju za usvajanjem novih znanja i iskustava. Već na početku karijere postao je prepoznatljiv po svom pozitivnom odnosu ne samo prema struci, nego i prema nauci. Tokom rada u Klinici završio je seminar „Statističke metode sa primenom u medicinskim istraživanjima“, kurs „Metodologija naučnog istraživanja u biomedicinskim naukama sa statističkom informatikom“ i seminar iz kardiologije u VMA. Kao rukovodioc, on je u periodu 1981–1985. godina realizovao naučni projekat *„Utvrđivanje sposobnosti za službu u JNA osoba sa hiperbilirubinemijom“* čiji je završni elaborat prihvatila Sanitetska uprava SSNO, 1986. godine.

Kako je govorio francuski jezik, potpukovnik asistent dr Marinković je početkom 1986. godine predložen za stručno usavršavanje u Francuskoj i naredbom načelnika Personalne uprave SSNO od 8. decembra 1986. upućen *u „Service d'hepatologie et unite de recherches de physiologie hepatique“* u Parizu, na usavršavanje po temi *„Savremena dijagnostika i lečenje hroničnih oboljenja jetre“*. Radilo se o Institutu za naučna istraživanja oboljenja jetre visokog svetskog naučnog ranga u sklopu bolnice u Božonu. Posle tromesečnog usavršavanja, dr Marinković je u svom

izveštaju zapisao da je, između ostalog, imao priliku da prisustvuje i učestvuje u radu sjajnog rukovodioca, lekara i pedagoga doktora *Jean – Pierre Benhamou*, jednog od najboljih stručnjaka Francuske na polju transplantacije jetre. Opisao ga je kao šezdesetogodišnjaka „mladalačkog izgleda, stalno nasmejanog lica, po svojim radnim navikama duboko odanog medicini i bolesnom čoveku, koji prosto ne zna granice redovnog radnog vremena, učitelja koji svojim iskustvom i stavom oduševljava mlade lekare i njegove starije kolege i prijatelje“.

Profesora Marinkovića su krasili ljudska blagost, smirenost i ozbiljnost u odnosu prema mlađima i podređenima, ali i velika znatiželja za sve njihove probleme i posebno snaga u traganju za najboljim rešenjima po pitanju dijagnostike i lečenja bolesnika. Nastojao je da najnovije svetske stavove iz oblasti dijagnostike i terapije infektivnih bolesti što je moguće pre primeni i u kliničkoj praksi. Njegova neobična energija i poštovanje mladih kolega su se u punoj meri ispoljili u nastavi, i to u više oblasti infektologije. Sa zadovoljstvom, velikim strpljenjem i svestrano je edukovao nekoliko generacija infektologa, posebno im ukazujući na pristup svim kategorijama bolesnika i nesebično im prenoseći lična znanja i iskustva. I to su njegovi učenici umeli da cene, poštujući njegovu ljubav prema teškom i mukotrpnom radu kliničkog lekara. Bio je izvođač svih vidova nastave koji su u to vreme bili zastupljeni na VMA. Imao je veći broj saopštenja na stručnim sastancima infektologa i hepatologa u zemlji i inostranstvu. Pored kliničkog rada i nastavne aktivnosti bio je aktivan i u publikovanju stručnih i naučnih radova. Objavio je oko 80 radova uglavnom u domaćim časopisima i zbornicima radova.

Profesor Marinković je bio je svestrana ličnost, tih, ozbiljan i nenametljiv, uprkos svojoj životnoj snazi i uspesima koje je postigao na ličnom i profesionalnom planu. Preminuo je 9. oktobra 2018. godine. Otišao je tiho i dostojanstveno iz naših redova, a nama, koji smo ga poznavali i poštovali, ostaće u trajnom sećanju.

Pukovnik
prof. dr Dragan Mikić

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Literatura

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

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Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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