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RISKY SEXUAL BEHAVIOR AND KNOWLEDGE ABOUT SEXUALLY TRANSMITTED INFECTIONS AMONG THE YOUNG PEOPLE

RIZIČNI SEKSUALNI ODNOSI I ZNANJA O SEKSUALNO PRENOSIVIM INFEKCIJAMA U POPULACIJI MLADIH

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Summary
Introduction. In recent years, there has been a growing global trend in the number of people with pathogens of sexually transmitted infections. There is a worldwide lack of data on the prevalence and incidence of sexually transmitted infections in the general population, especially among the high school students. **Material and Methods.** We conducted a cross sectional study on attitudes and knowledge of young people about sexually transmitted infections. The survey population included young people aged 14 to 20 years attending a high school in the city of Novi Sad. The data were collected through a questionnaire. **Results.** The percentage of students with signs and symptoms of sexually transmitted infections was higher among the students who did not use a condom during the last sexual intercourse compared to the students who used a condom. **Conclusion.** Young people engaging in risky sexual behavior showed better knowledge about sexually transmitted infections in comparison to young people who did not engage in risky sexual behavior.
Key words: Sexually Transmitted Diseases; Sexual Behavior; Adolescent; Young Adult; Condoms; Health Knowledge, Attitudes, Practice; Schools; Surveys and Questionnaires; Risk-Taking

Sažetak
Uvod. Poslednjih godina na svetskom nivou prisutan je trend porasta broja ljudi koji su inficirani patogenima koji uzrokuju seksualno prenosive infekcije. Problem koji postoji širom sveta je nedostatak suficijentnih podataka prevalencije i incidencije seksualno prenosivih infekcija u celokupnoj populaciji, a pogotovo u srednjoškolskoj populaciji. **Materijali i metode.** Sproveli smo studiju preseka stavova i znanja mladih o seksualno prenosivim infekcijama. Populaciju koja je obuhvaćena istraživanjem činili su mladi starosti između 14 i 20 godina. Za uzorak su uzeti učenici jedne gimnazije u Novom Sadu. Podaci su prikupljeni pomoću upitnika. **Rezultati.** Kod većeg procenta učenika koji nisu koristili kondom tokom poslednjeg seksualnog odnosa uočeni su simptomi i znakovi uobičajeni za seksualno prenosive infekcije u poređenju sa populacijom studenata koji su koristili kondom. **Zaključak.** Mladi kod kojih je otkriveno postojanje rizičnog seksualnog ponašanja imaju bolje znanje o polno prenosivim infekcijama u odnosu na mlade ljude koji ne praktikuju rizičan seks.
Ključne reči: polno prenosive bolesti; sekusalno ponašanje; adolescent; mlada osoba; kondomi; znanje o zdravlju, stavovi, praksa; škole; ankete i upitnici; rizično ponašanje

Introduction

In recent years, there has been a growing global trend in the number of people with sexually transmitted infections (STIs) and the number of people with clinical manifestations of STIs. The number of people seeking medical help due to problems caused by STIs has also been on the rise for years [1–10]. There are more than 20 species of STI-induced microorganisms. Many of them are susceptible to different types of antibiotics. However, the major problem is how to deal with the increasing number of bacteria causing STIs that are resistant to standard antibiotic therapy

[10]. Epidemiological studies have shown that there are major differences in the prevalence of STIs between populations of different countries, regions within the same state, urban and rural populations, even among persons belonging to the same socioeconomic groups within the same territory. In general, the prevalence of STIs is higher among residents of urban areas, among non-married people and among people aged 15 – 35 [10–14]. Great medical problems are complications due to untreated and inadequately treated STIs, such as pelvic inflammatory disease, ectopic pregnancy, prostatitis, epididymitis, sterility and others. Another worldwide problem, in-

cluding our country, is that there is insufficient data on the prevalence and incidence of STIs at the level of the entire population, and especially in the high school population. In general, accurate data on the overall prevalence and incidence of STIs, currently do not exist in any country. In some countries of the world, including Serbia, there is a program of passive epidemiological surveillance of STIs, which is mainly based on data sent by family doctors and clinical doctors to the appropriate service. A particular specificity of studies on the prevalence and incidence of STIs is that most STIs are usually asymptomatic in the beginning. Of the total number of people who suffer from STIs, only a small part seeks medical attention, and even fewer are diagnosed with STI. A research carried out by different experts estimated that around 340 million people have been infected, symptomatically or asymptomatically with some potentially curable STI [10–14]. A large number of countries have an increase in the number of people with STI, but more and more often, they suffer from more than one STI at the same time. It is particularly worrying that in most European countries, including Serbia, the number of people infected with human immunodeficiency virus (HIV), as well as the number of people who have HIV infection and syphilis at the same time, is growing or has a stable trend without the tendency to fall. On the other hand, a research conducted in the last several years in the European Union has shown that in most European countries the number of people who are asymptomatically, but also symptomatically, infected with Chlamydia trachomatis is stable, and that the largest number of infected persons of both sexes are under the age of 25. In addition, as yet another equally important problem, there is even less data and studies related to the attitudes and knowledge of young people about STIs, although persons aged 15 – 25 are mostly affected with these infections [12–15]. For example, studies have shown that of the total number of women diagnosed with chlamydia, 24.1% – 27% are adolescents [9, 12]. Genital warts are a very widespread type of STI, caused by human papillomavirus (HPV); some types are considered the main causes of cervical cancer and penile carcinoma. The highest incidence of genital warts in the female population is aged between 15 – 24 years, and 20 – 29 years in the male population. People with genital warts are at greater risk of getting cervical or penile cancer compared to the rest of the population. The prevalence and infection with Herpes simplex type 2 virus is also high among young people. This infection is not curable and the special problem is that the lesions caused by this virus on the skin of genital organs increase the risk of HIV infection. A positive growing trend of people with STI in underdeveloped and developing countries is mainly due to the growing poverty of most of the population, prostitution, poor education, migration from rural to urban areas, psychoactive substances and alcohol abuse resulting in higher incidence of risky sexual behavior, rape and unwanted pregnancies. The aforementioned trend is also the consequence of the lack of developed health services and virtually non-existent prevention programs. On the other hand, in developed countries, the increase in incidence and prevalence of STIs is associated with increased abuse of psychoactive substances, alcohol and all the consequences that result from it – unprotected sex and more frequent sexual activity with more sexual partners [13–19].

The aim of the research was to gain insight into the attitudes and knowledge of the young population on STIs and to find out if young people with more knowledge on STIs rarely engage in risky sexual behavior.

Material and Methods

The aim of this cross-sectional research was to investigate the attitudes and knowledge of young people about STIs. The survey included young people aged 14 to 20, of which 99% were 15 to 19 years old. The entire sample included in the research were students of a grammar school in Novi Sad. Only students of this grammar school could participate in the investigation. The researchers excluded students who were not regular students of the previously mentioned grammar school. The total number of students in the school is 1.096. The survey covered a total of 338 students in 14 classes. The students were randomly selected as follows: 3 classes of the first, second and fourth grades, and 5 classes of the third grade. The total number of respondents who attended the first year was 92 (32.18% of the total number of pupils in the first grade), 68 students of the second grade (25.37%), 115 pupils of the third grade (43%) and 64 students of the fourth grade (23.53%).

The hypothesis was tested based on data obtained by processing and comparing data from two groups of respondents. The participants were divided based on their answers to the question whether they used a condom during the last sexual intercourse. The first group included students who used a condom (a total of 89 students), and the second group did not use a condom (a total of 18 students). The average age of the respondents in the first group was 16.97 years, and in the second it was 17.17 years. Both groups had an equal number of males and females. Data gathered from students who answered that they did not have sexual intercourse were not analyzed.

The data collection was done using printed questionnaire forms. All respondents received the same questionnaire. Respondents were given 10 minutes to independently complete the questionnaire. The questionnaire contained 31 questions. At the beginning of the questionnaire, respondents were informed about the reasons for data collection, they were given instructions for filling in the questionnaire, as well as

a notification that the questionnaire was completely anonymous. The questionnaire contained both open and closed questions. The closed type questions were: multiple checklist questions, multiple rating scale questions, multiple alternative questions, dichotomous questions. The first nine questions were selected to collect demographic data about the respondents. From 10th to 18th questions were questions on the knowledge of respondents about STIs. The rest of the questions (from 19 to 27) referred to the views of respondents about STDs, interest, the source of information on STDs, who they would turn for advice, etc. The last part of the inquiry included questions whether the respondents had a sexual relationship, whether they used a condom during the last sexual intercourse, how many sexual partners they had during the past year, and how old they were when they had their first sexual intercourse.

The data used in the survey were obtained by chi square test, t-test, mean values and proportions. The data obtained by the first two tests were processed using EduStat. 2.01.

Results

Compared to the students who used condoms, a higher proportion of students who did not use condoms during their last sexual intercourse listed signs and symptoms of common STIs such as redness and burning sensation in the genital area, pain in the area of genital organs and burning sensation during miction, appearance of blisters and rash on genitals, as well as inflammation of the genital organs (pain, redness, swelling) **(Tables 1 and 2)**. Only 16.95% of those in the group who used condoms listed three or more accurate signs and symptoms of STIs (including symptoms/signs of acute HIV-infection, acquired immune deficiency syndrome (AIDS) and hepatitis) **(Table 3)**. In the second group, who did not use condoms, the percentage of respondents who listed three or more exact signs and symptoms of STIs was 38.39%, which is about 2.3 times higher than in the first group. On the other hand, in both examined groups of students, there was an equal percentage of respondents who did not list any exact symptom/sign of STIs (including symptoms/signs of acute HIV-infection, AIDS and hepatitis) accounting for about 55%. Statistical analysis of the data about the association between STIs and genital carcinomas provided by respondents from both groups showed that there was no statistically significant difference between the compared groups. Statistical analysis of the data about the knowledge of STI transmission provided by respondents of both observed groups (urban and rural population) showed that there was no statistically significant difference between the compared groups. Statistical analysis of the data about the knowledge about the possibility to transmit STI by oral sex without condoms of both groups showed that there was no statistically significant difference between the compared groups. Statistical analysis of the data about the knowledge of asymptomatic STIs showed that there was no statistically significant difference between the groups compared. Statistical analysis of the data about the attitude of the respondents about the implementation of sex education into school programs showed that there was no statistically significant difference between the compared groups. The same was found about counseling on HIV and other STIs in Novi Sad. Most students in both groups did not know whether there was counseling or where to get it (80% of the first and 87.5% of the respondents in the second group). The chi square test was used for data processing.

Statistical analysis of the data about the attitude of the respondents of both groups about whether they knew enough about the types and modes of STD transmission showed that there was no statistically significant difference between the compared groups. Statistical analysis of the data about a desire of the respondents to learn more about STIs showed that there was no statistically significant difference between the groups. The same was found about the desire of respondents to learn about professional websites on the Internet where they could get more information about STIs. The chi square test was used for data processing. Statistical analysis of the data about the attitude of the respondents towards stigmatization of persons with STIs showed that there was no statistically significant difference between the groups compared. Statistical

Table 1. Knowledge of sexually transmitted infections (STI) among young people
Tabela 1. Poznavanje sekusalno prenosivih infekcija (SPI) kod mladih

Sexually Transmitted Infection (STI) *Sekusalno prenosive infekcije (SPI)*	Gonorrhea *Gonoreja*	Syphilis *Sifilis*	HIV infection *HIV infekcija*	Hepatitis B and C *Hepatitis B i C*	Genital herpes *Genitalni herpes*	Chlamydia *Hlamidija*
Percentage of respondents who identified STIs in the population who use condoms (%)/*Procenat ispitanika koji su zaokružili SPI u populaciji koja je koristila kondom (%)*	27.12	83.05	94.91	37.29	32.2	30.51
Percentage of respondents who identified STIs in the population not using condoms (%)/*Procenat ispitanika koji su zaokružili SPI u populaciji koja nije koristila kondom (%)*	55.56	94.44	100	61.11	38.89	55.56

Legenda: HIV – virus humane imunodeficijencije

Table 2. Percentage of respondents who identified the signs and symptoms of sexually transmitted infections, other than HIV
Tabela 2. Procenat ispitanika koji su identifikovali simptome i znakove seksualno prenosivih infekcija, osim HIV-a

Symptoms and signs of sexually transmitted infection (STI) *Simptomi i znaci seksualno prenosive infekcije (SPI)*	Percentage of respondents who indicated the symptoms/signs of STIs in the group that used a condom during the last sexual intercourse (%)/*Procenat ispitanika koji su naveli simptome/znake SPI u grupi koja je koristila kondom u toku poslednjeg seksualnog odnosa (%)*	Percentage of respondents who indicated the selected symptoms/signs of STIs in the group that did not use a condom during the last sexual intercourse (%)/*Procenat ispitanika koji su naveli simptome/znake SPI u grupi koja nije koristila kondom u toku poslednjeg seksualnog odnosa (%)*
Genital itching/*Svrab genitalne regije*	25.42	33.33
Genital tingling/*Osećaj peckanja u genitalijama*	5.08	11.11
Pain in the genitals/*Bol u genitalijama*	6.78	5.56
Genital pain during urination *Pojavljivanje bola u genitalnim organima tokom mokrenja*	1.69	5.56
Tingling sensation when urinating *Osećaj peckanja prilikom mokrenja*	0	5.56
Blood during urination/*Pojava krvi u urinu*	3.39	0
Genital redness/*Crvenilo genitalija*	5.08	16.67
Genital lesions/*Pojava rana na genitalijama*	6.78	0
Genital rash/*Pojava osipa na genitalijama*	3.39	5.56
Genital blisters/*Pojava plikova na genitalijama*	5.08	11.11
Genital herpes/*Genitalni herpes*	1.69	0
Genital swelling/*Oticanje polnih organa*	5.08	5.56
Vaginal or penile discharge *Pojava pojačanog vaginalnog sekreta ili sekreta iz penisa*	3.38	0
Genital bleeding/*Krvarenje iz genitalija*	1.69	5.56
Unpleasant genital odor/*Neprijatan miris genitalija*	1.69	0
Genitals changes/*Genitalne promene*	1.69	0

Legenda: HIV – virus humane imunodeficijencije

Table 3. Percentage of respondents who listed the symptoms/signs of acute HIV infection
Tabela 3. Procenat ispitanika koji su naveli gore navedene simptome/znake akutne HIV infekcije

Symptoms and signs of acute HIV infection *Simptomi i znaci akutne HIV infekcije*	Percentage of respondents who listed the symptoms/signs of HIV infection in the group that used a condom during the last sexual intercourse (%)/*Procenat ispitanika koji su naveli simptome/znake u grupi koja je koristila kondom u toku poslednjeg seksualnog odnosa (%)*	Percentage of respondents who indicated the signs/symptoms of HIV infection in the group that did not use a condom during the last sexual intercourse (%)/*Procenat ispitanika koji su naveli simptome/znake u grupi koja nije koristila kondom u toku poslednjeg seksualnog odnosa (%)*
Fever/*Temperatura*	1.69	0
Exhaustion/*Malaksalost*	6.78	5.56
Headache/*Glavobolja*	1.69	5.56
Nausea/*Mučnina*	3.39	5.56
Vomiting/*Povraćanje*	0	0
Diarrhea/*Dijareja*	0	0
Muscle pain/*Bol u mišićima*	3.39	0

Legenda: HIV – virus humane imunodeficijencije

analysis of the data about the attitude towards the personal responsibility of an individual when it comes to STIs showed that there was no statistically significant difference between the groups. Statistical analysis of the data about the attitude of young people about the responsibility of parents, school and the general public towards the education of young people about STIs showed that there was no statistically significant difference between the groups compared. The chi square test was used for processing all mentioned data.

Table 4. Age of the examinees at first sexual intercourse
Tabela 4. Prikaz starosti ispitanika prilikom ulaska u prvi seksualni odnos

The age at first sexual intercourse *Uzrast pri stupanju u prvi seksualni odnos*	The number of students who used a condom at the last sexual intercourse *Broj učenika koji su koristili kondom prilikom poslednjeg seksualnog odnosa*	The number of students who did not use a condom during the last sexual intercourse *Broj učenika koji nisu koristili kondom prilikom poslednjeg seksualnog odnosa*
12	0	1
13	2	0
14	5	2
15	8	4
16	19	5
17	18	3
18	2	2

Table 5. The number of sexual partners of the respondents in the last calendar year
Tabela 5. Broj seksualnih partnera koje je ispitanik imao tokom posljednje kalendarske godine

The number of sexual partners in the past year *Broj seksualnih partnera u toku prošle kalendarske godine*	Percentage of young people who used a condom during the last sexual intercourse (%)/ *Procenat mladih koji su koristili kondom u toku poslednjeg seksualnog odnosa (%)*	Percentage of young people who did not use a condom during the last sexual intercourse (%) *Procenat mladih koji nisu koristili kondom u toku poslednjeg seksualnog odnosa (%)*
0	0	11.11
1	58.62	27.78
2	25.14	33.33
3	8.62	11.11
4	0	0
5	0	5.55
>5	8.62	11.11

The data from **Table 4** were statistically processed using the T-test. Since Ua = -1.96 < U0 < Ua = +1.96, the zero hypothesis was accepted with a probability of 95%. It indicated that the difference in the average age at first intercourse between the group of students who used a condom during the last sexual intercourse and the group that did not use a condom was not statistically significant. The average age at first intercourse was almost identical in students of both groups and it was 15.96 years in the first group, and 15.7 in the second group. The average number of sexual partners per respondent in the past year was 1.84 in the group of young people who used condoms, and 2.22 in the group who did not use condoms **(Table 5)**.

Discusion

Differences in knowledge and attitudes of young people about STIs depend on a number of factors. Among other things, they can be explained by people's belonging to a particular socio-economic group [20–22]. The socio-economic group is defined by 3 parameters, the person's level of education, his occupation, and income level [18, 23]. Although this study did not examine the income level of the respondents and people who supported them, the common thing among them was that they attended the same grammar school, so they had the same level of

education. However, some studies have shown that there are significant differences within the same socio-economic group in knowledge about STIs, but also in the level of individual activity and their tendency to engage in risky sexual behavior [7]. The aforementioned differences can be the result of individual personality traits, but also of possible family violence and other traumas that a person experienced during childhood or youth [24]. There are studies that found that those who were abused or abandoned in childhood engaged in sexual intercourse earlier and were more likely to change sexual partners and have sex without a condom than their peers who had not had such traumatic experiences [24, 25]. In the conducted research, the average age of the first sexual intercourse ranges from 12 to 18 years of age, and the number of sexual partners during a year varies greatly - from no sexual partner to more than five partners. Attention should be paid to the great influence young people have on their peers, especially when it comes to sexual activity [26]. Young people are more open to asking questions about STIs and are more willing to accept information from their peers about the types or ways of STI transmission. In addition, public figures who are being recognized by young people as role models and whose lifestyle and behavior they follow also have a significant role in developing attitudes about a number of issues, including the use of mechanical protection during sexual intercourse. The

importance of public personalities in adopting healthy forms of behavior in adolescents and young people has long been recognized and exploited for drug prevention, smoking and recent STI prevention programs [14]. In our country, there is a need for famous people to promote the use of condoms as a form of protection from STIs and unwanted pregnancy. The need for further promotion and dissemination of knowledge about the importance of using condoms is also reflected in the example of this study, since it has been established that out of the total number of young people covered by the survey who stated that they had sexual intercourse, 23% claimed they did not use a condom during their last sexual intercourse. This percentage is far below the percentage of young people who do not use condoms in sexual relationships in other countries, for example Malaysia or Jamaica [13, 14]. Generally, most young people globally do not use condoms during sexual intercourse [13, 14, 19].

Subjects who claimed that they did not use a condom during their last sexual intercourse, listed more accurate symptoms of classic STIs such as itching, redness and pain in the genital area, pain during urination, compared to those students who used condoms. Furthermore, most of the symptoms mentioned are the usual clinical manifestations of urogenital infections with chlamydia trachomatis, mycoplasma hominis or ureaplasma. Also, listing chlamydia as a STI can be the result of better knowledge of respondents and of fear or concern for their own health resulting from unprotected sexual intercourse. People who do not use condoms are at a greater risk of getting STIs, especially those that are most widespread, and in case of Vojvodina, they are primarily genital warts and urogenital infections with mycoplasma. A common occurrence is that, when a person gets an infection, he or she asks and learns more about it from those who are healthy, and this might be the reason why more students who did not use condoms answered questions about the types of STIs and their clinical manifestations correctly.

Urethritis is the most common manifestation of STIs in male individuals. During the last 20 years, the incidence of gonococcal urethritis (GU) has declined significantly in developed countries, but the incidence of nongonococcal urethritis (NGU) has remained high and has not changed. In most environments, chlamydia trachomatis accounts for about 40% of detected and treated NGU cases. Herpes simplex virus and trichomonas vaginalis account for much lower percentages compared to chlamydia trachomatis. One of the possible etiological agents of NGU is ureaplasma urealyticum, as well as coliform bacteria (e.g. escherichia coli). The diagnosis of male urethritis, in most cases of clinical practice, does not include specific tests except for neisseria gonorrhoeae and

chlamydia trachomatis. Inflammation of the lower urinary and genital tracts in females is usually caused by coliform bacteria, mostly escherichia coli. However, STI agents should also be taken into account, as the infection with escherichia coli manifests the same as STIs.

It is generally accepted that men are more inclined to engage in sexual intercourse without using condoms, with more sexual partners, and have sex with prostitutes than women [27]. The research showed that there is an equal number of young people of both sexes who do not use condoms as a protection against STIs. The most common reason they give for not using condoms during a sexual intercourse is that they have a permanent sexual partner who they believe is faithful and has no STI, because they look healthy and have no changes on the sexual organs [28]. Some of the girls whose partners do not use a condom during sexual intercourse think that they do not need it because they use oral contraceptives [28].

An interesting outcome of the study is that the best known STI is HIV infection, while many students have never heard of other STIs, such as genital chlamydiae or hepatitis B and C. This result is, firstly, due to lectures and workshops on HIV infection and prevention of HIV/AIDS. Secondly, in the public, primarily on television and radio, of all STIs, the most talked about is HIV infection, although this infection is not widespread in the population of Vojvodina. Most young people are interested in learning more about STIs and are ready to attend classes on this topic and use the Internet as a health care tool.

There is still a pronounced stigmatization and harsh criticisim of persons with STIs throughout the world. Such persons are often characterized as irresponsible, promiscuous and sexually hyperactive, and thus they are believed to deserve to have STIs. [6] As far as our research is concerned, most young people think that people with STIs should not be disgruntled, but that people are often, not completely, to blame for getting STIs. Changes in attitudes towards STIs are mainly the result of education, but also personal experience of individuals. The progress made in reducing stigma towards persons suffering from STIs is insufficient and further efforts need to be made by individuals, non-government and government organizations in order to fight to reduce stigma associated with STIs.

Conclusion

Young people who have been exposed to risky sexual behavior (sex without a condom) showed more knowledge about sexually transmitted infections than young people who did not engage in risky sexual behaviors.

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EFFECTS OF DIFFERENT TYPES OF TRAINING ON WEIGHT LOSS

UTICAJ RAZLIČITIH TIPOVA TRENINGA NA GUBITAK TELESNE TEŽINE

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Summary

Introduction. The difference between 24-hour daily energy intake and total daily energy expenditure determines whether we lose or gain weight. The resting metabolic rate is the major component of daily energy expenditure, which depends on many different factors, but also on the level of physical activity. The aim of the study was to determine anthropometric and metabolic parameters of athletes engaged in different types of training, to compare obtained results and to examine whether there are statistically significant differences among them. **Material and Methods.** The study included a total of 42 young male athletes divided into two groups. The first group included 21 athletes who were predominantly engaged in aerobic type of training, and the other group of 21 athletes in anaerobic type of training. Anthropometric measurements were taken and resting metabolic rate was assessed using the indirect calorimetry method. The results were statistically analyzed and the differences in parameters between the two groups were compared. **Results.** Statistically significant differences were established in total body mass, amount of fat-free mass and muscle mass, body mass index, as well as in the relative metabolic indices between two groups of subjects. **Conclusion.** The percentage of fat-free body mass has the greatest impact on the resting metabolic rate. The rate of metabolic activity of this body compartment is higher in athletes engaged in aerobic than in athletes engaged in anaerobic type of training.
Key words: Weight Loss; Exercise; Athletes; Anthropometry; Calorimetry, Indirect; Energy Metabolism; Basal Metabolism; Oxygen Consumption; Resistance Training; Anaerobic Threshold

Sažetak

Uvod. Razlika između 24-časovnog dnevnog energetskog unosa i ukupne dnevne energetske potrošnje određuje da li će doći do porasta u telesnoj težini ili njenog smanjenja. Najveći udeo u dnevnoj potrošnji čini energetska potrošnja mirovanja koja zavisi od mnogo različitih faktora ali takođe i od nivoa fizičke aktivnosti. Cilj ovog rada bio je da se odrede vrednosti antropometrijskih i metaboličkih parametara sportista na različitom tipu treninga, uporede dobijeni rezultati i ispita da li postoje statistički značajne razlike između njih. **Materijal i metode.** Ispitivanje je obuhvatilo ukupno 42 mladih sportista muškog pola podeljenih u dve grupe. Prvu grupu je činio 21 sportista pretežno na aerobnom tipu treninga, a drugu grupu je činio 21 sportista na anaerobnom tipu treninga. Svim ispitanicima su mereni antropometrijski parametri i energetska potrošnja mirovanja kao metabolički parametar metodom indirektne kalorimetrije. Dobijeni rezultati su statistički obrađeni i upoređene su razlike u parametrima između dve grupe sportista. **Rezultati.** Prilikom obrade antropometrijskih i metaboličkih parametara utvrđeno je da postoje statistički značajne razlike u telesnoj masi, količini bezmasne i mišićne mase, indeksu telesne mase kao i u relativnim metaboličkim pokazateljima dve grupe ispitanika. **Zaključak.** Najveći uticaj na energetsku potrošnju mirovanja ima procenat telesne mase koji ne sadrži masti, tj. bezmasna masa tela. Stopa metaboličke aktivnosti ovog telesnog kompartmana je veća kod sportista na aerobnom u odnosu na sportiste na anaerobnom tipu treninga.
Ključne reči: gubitak težine; vežbanje; sportisti; antropometrija; indirektna kalorimetrija; energetski metabolizam; bazalni metabolizam; potrošnja kiseonika; trening snage; anaerobni prag

Introduction

Metabolism is the set of all biochemical reactions which are happening in the cells of an organism that are involved in catabolism and anabolism of organic substrates. The term metabolism refers to the amount of heat generated during these processes. The minimal amount of energy required to sustain basic vital functions is known as the basal metabolic rate (BMR) and it accounts for 50–70% of total daily energy expenditure (TDEE) [1]. The largest component of daily energy expenditure is indispensable for processes such as circulation, breathing, nervous system functioning etc. The second largest component of TDEE is physical activity related energy expenditure that accounts for 15 to 30% depending on the type and intensity of physical activity **(Graph 1)**. The last component of TDEE is the thermic effect of food (TEF), accounting for approximately 10% and it represents the additional energy used for digestion and absorption of nutrients, as well as storage and synthesis of organic compounds [2].

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In scientific literature, the term resting metabolic rate (RMR) is often incorrectly used instead of basal metabolic rate (BMR), whose values are approximate to those of basal metabolism, but the method used to acquire it is less demanding and easier to perform [2]. Since the values of RMR are ~10% higher than of BMR [3], this component is certainly the largest contributor to TDEE.
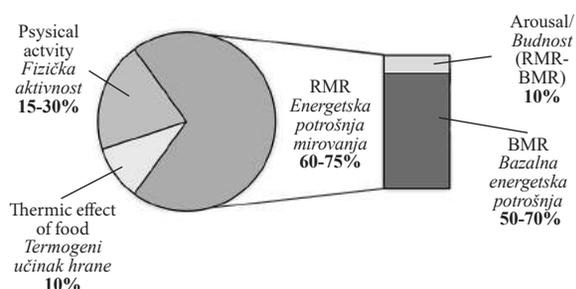
Graph 1. Components of total daily energy expenditure (TDEE) - modified according to Hall and Katch
Grafikon 1. Komponente ukupne dnevne energetske potrošnje (TDEE) – modifikovano prema Halu (Hall) [1] i Kaču (Katch) [2]

Many studies have shown that the most important factor affecting the BMR (i.e. RMR) is the part of the body mass that does not include fat, that is, the fat-free mass (FFM) [4]. Considering that FFM is mainly composed of muscle mass and that it accounts a lot in the total body weight, it can be concluded that the muscles significantly account for resting energy expenditure [5, 6]. So, regardless of variations in the amount of adipose tissue in an individual, if there are no changes in FFM compartment, the RMR remains approximately the same as before and amounts to 60 - 75% of its daily calories expended **(Graph 1)**. The RMR value is also affected by the age and gender of the individual, hormone levels, circadian rhythm, body temperature, physical activity, genetics and malnutrition. In the course of aging, RMR progressively diminishes, mainly due to the reduction in the amount of skeletal muscle mass and mass of internal organs, gradual decrease of metabolic activity of individual organs as well as due to an increase in the proportion of fat mass which generally has a lower

metabolic rate [7]. Gender differences in RMR values are also caused by a smaller amount of muscle mass and a higher amount of fat mass in women. Hormonal imbalance can also greatly affect RMR, increasing it by as much as 50 – 100% in case of thyroid hormones and 15 – 20% in case of elevated growth hormone levels, while increased testosterone levels may increase RMR values by 10 – 15% [1]. A rise in body temperature increases RMR, while sleep lowers it, due to the reduction of skeletal muscle tone and the effects of the vegetative nervous system [8]. Also, it is established that situations with reduced energy intake lead to a decrease in RMR [9, 10].

Physical activity changes the body composition, i.e. it changes the proportion of fat and fat-free mass in overall body weight and thus brings the athlete a more favorable ratio of these two components, compared to non-athletic individuals. Body composition is important to the majority of athletes, particularly because the ratio of fat to fat-free mass affects their sports performance [11, 12]. Body composition can be determined indirectly (by measuring skinfold thicknesses, bioelectrical impedance analysis method, etc.) or directly, i.e. by directly measuring the fatty and non-fatty tissue using imaging methods (magnetic resonance imaging, computed tomography, etc.) [13, 14].

Physical activity leads to an increase in total daily energy expenditure by a triple effect: first, by energy expenditure spent during physical activity itself, second, by promoting higher excess post-exercise oxygen consumption (EPOC) after physical activity and third, by long-term elevation of RMR [15]. The RMR increases by increasing fat-free mass, but also by physiological processes induced by physical activity. Both of these effects can occur as a consequence of long-term training but also as a consequence of a single training session. After a single training session, there is a transient increase in RMR, which actually represents an EPOC during which the additional energy expenditure is used to replenish the anaerobic sources of energy [16]. Some studies have found that long-term training, either aerobic or anaerobic, leads to an increase in RMR, while other studies did not. In fact, in certain number of examinees, the RMR actually decreased after long-term training, but the cause of this effect is unknown [17, 18]. It is considered to be a result of down-regulation of uncoupling protein 3 (UCP3) whose role is to improve the mechanical efficiency of muscles during exercise [19]. The other possible cause of this effect could be the reaction of the organism to exposure to excessively strenuous physical activity [16]. Zurio et al. pointed out that muscle energy consumption depends on the type of muscle fibers [5], while the representation of certain muscle fibers is mainly determined by the type of physical activity through the process of neuromuscular adaptation to training [20]. Resistance training will develop the energy capacity in the direction of anaerobic energy system, while the continuous (steady-state) training will generally develop energy capacity in the direction of aerobic energy system. Fast-twitch IIa (fast oxidative-glyco-

lytic) and fast-twitch IIx (fast-glycolytic) fibers have a greater amount of myofibrils and the ability to generate more adenosine triphosphate (ATP) that allows them to produce rapid and powerful muscle contractions that are crucial for dominantly anaerobic sports. Slow-twitch I fibers (slow-oxidative), on the other hand, are better vascularized and contain higher amounts of mitochondria and myoglobin, thus getting most of their energy out of aerobic sources [21].

The aim of this study was to assess values of anthropometric parameters and parameters of resting energy expenditure of athletes engaged in different types of training and to determine whether different types of training, practiced by athletes for at least two years back, affect RMR, as well as to determine whether the differences in RMR and anthropometric parameters are statistically significant among these athletes.

Material and Methods

Participants
The study included a total of 42 young, male Caucasian athletes who were divided into two groups. The first group included 21 athletes who were engaged predominantly in aerobic training, while the second group of 21 athletes was predominantly engaged in anaerobic type of training. Sports were classified according to Mitchell classification of sports [22]. The inclusion criteria were: athletes engaged in a certain type of training for at least two years back, three times a week, and 90 minutes per training session. Exclusion criteria were: endocrine disorders, acute infectious disease, and use of substances that could affect the level of RMR. Prior to the study, after receiving clear information about the study protocol, all of the participants gave their informed consent. All of the measurements were performed at the Laboratory of Functional Diagnostics of the Department of Physiology, Faculty of Medicine. The research was approved by the Ethics Committee for Clinical Research of the Faculty of Medicine.

Anthropometric measurements
Anthropometric assessments included the following measurements: total body weight (BW), height (Ht), skinfold thicknesses, and body circumferences. The BW was measured on a medical beam scale (precision of 0.1 kg) with subjects lightly dressed. The Ht was measured using a stadiometer with a precision of 0.1 cm; barefoot participants were standing heels together and their heads in the Frankfurt plane. Skinfold thicknesses (pectoral, subscapular, midaxillary, biceps, triceps, abdominal, mid-thigh, medial calf, suprailiac, supraspinale) were measured on the right hand side using the Harpenden caliper with the precision of 0.2 mm, while the circumferences (forearm, relaxed arm, flexed and tensed arm, chest, gluteal, mid-thigh and calf) were measured using a flexible tape measure with the precision of 1 mm, also on the right hand
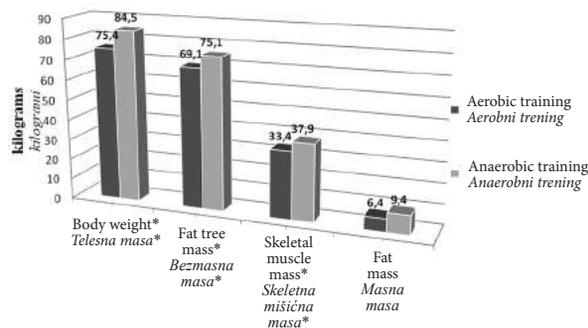
Graph 2. Differences in body composition of two examined groups (* p < 0.05)
Grafikon 2. *Razlike u telesnom sastavu dve ispitivane grupe (* p < 0,05)*

side [23, 24]. Also, biepicondylar femur and humerus breadths were assessed using Holtain Bicondylar Vernier caliper with a precision of 0.1 cm.

From the obtained parameters, density of the body (Db) was first calculated using the Jackson and Pollock equation [25] for male athletes aged ≥ 20 years:

$$Db = 1,112 - 0,00043499*(\Sigma 7SKF) + 0,00000055*(\Sigma 7SKF)^2 - 0,00028826*(age),$$

with $\Sigma 7SKF$ representing summed up values of pectoral, midaxillary, triceps, subscapular, abdominal, suprailiac and mid-thigh skinfold thickness.

Furthermore, the percentage of fat mass (FM%) out of Db was calculated using the Siri equation [26]:

$$FM (\%) = ((4,95/Db)-4,5)*100$$

Based on the calculated FM (%), the absolute value of the amount of the body FM (kg) was determined. Fat-free mass (FFM) was calculated by subtracting the FM (kg) from the total BW (kg) (FFM = BW - FM).

Lee et al. conducted an imaging study of 244 participants aged 20 – 81, proposing a predictive equation for estimation of the total skeletal muscle mass (SM) (kg), based on anthropometric measurements (height, skinfold thickness, limb girths, sex, age and race (so called, skinfold-circumference model) [27], which we used to estimate the total SM of an athlete:

$$SM (kg) = Ht \times (0.00744 \times CAG^2 + 0.00088 \times CTG^2 + 0.00441 \times CCG^2) + 2.4 \times sex - 0.048 \times age + race + 7.8$$

with: Ht - height; CAG - corrected arm girth; CTG - corrected thigh girth; CCG - corrected calf girth; sex - 1 for males and 0 for females; race - -2.0 for Asians, 1.1 for African Americans and 0 for Caucasians or Hispanics. Corrected limb girths = limb girth - (π x skinfold of proper limb).

Resting metabolic rate measurement
Nowadays, indirect calorimetry is assumed to be a gold standard for measuring RMR [28]. It is more convenient to perform compared with direct calorimetry, which measures the amount of heat generated by using much more sophisticated equipment. Indicators of resting energy expenditure were assessed by evaluating RMR using the FitmatePro (Cosmed, Rome) at the Laboratory of Functional

Diagnostics of the Department of Physiology, Faculty of Medicine. The RMR was measured in the morning in a thermoneutral environment, i.e. air temperature of 22 – 25° C. The subjects were instructed in advance about the methodology of the study and it was explained to them which conditions were necessary to avoid that might influence RMR values: recent physical activity, thermic effect of food, acute diseases or some anabolic/catabolic substances. The most important criteria required athletes not to engage in any intense physical activity 36 hours prior to the measurement, to exclude effects of excess post-exercise oxygen consumption on RMR. It was also required not to consume food, alcohol, caffeine or nicotine 12 hours before the testing. To avoid effects of recent physical activity, the participants came to the lab either by a motor vehicle or on foot, walking leisurely and slowly. The measurement was performed under the conditions of psychophysical relaxation and after the participants had a good night's sleep. Before the measurement was performed, the subjects had been resting in a seated position for at least 15 minutes and then they lied down for 15 minutes with a mask on their faces, through which the FitmatePro measured the oxygen consumption. From these values, the integrated software calculated the values of RMR.

Afterwards, relative indices of resting energy expenditure were calculated by dividing the obtained values of RMR with total BW, FFM and total SM mass (RMR/BW, RMR/FFM and RMR/SM).

The obtained results were statistically processed in Microsoft Excel 2013 and Jeffrey's Amazing Statistical Program (JASP, ver. 0.8.0.1.). Pearson's level of correlation (r) of RMR values with different anthropometric parameters was calculated and Student's t-test was used to determine if there were any statistically significant differences between the anthropometric and metabolic parameters of two examined groups. The value of $p < 0.05$ was considered as statistically significant.

Results

The mean age of the participants (n = 42) was 21.76 ± 2.32 years, mean height 182.4 ± 6.5 cm, total BW 79.98 ± 12.09 kg, body mass index (BMI) 24 ± 3.14 kg/m², body fat percentage $9.33 \pm 5\%$, total SM percentage $44.8 \pm 3.42\%$, and mean RMR was 2461.81 ± 394.38 kcal/24 h. No statistically significant differences were found in age, height, body fat percentage, muscle mass percentage and RMR ($p > 0.05$) between the two groups of athletes, while the parameters of total BW, BMI, FFM and total SM mass showed statistically significant differences between the groups ($p < 0.05$) **(Table 1)**. Significantly higher values were found in the group of athletes engaged in anaerobic training in the parameters of body mass (84.52 ± 13.73 vs. 75.43 ± 8.25 kg), BMI (25.29 ± 3.64 vs. 22.71 ± 1.86 kg/m²), FFM (75.14 ± 8.95 vs. 69.06 ± 6.33 kg) and total SM mass (37.93 ± 5.08 vs. 33.36 ± 3.07 kg). These differences in body composition are presented in **Graph 2**.

The level of correlation between RMR and basic anthropometric parameters, skinfold thickness and body circumference was analyzed in each group as well as in all the participants **(Table 2)**. Since the subjects in our investigation were of the same age, no statistically significant correlation regarding their age was found ($p > 0.05$). The statistically most significant ($p < 0.001$) correlation was between RMR and FFM, SM mass and total BW (r = 0.563, 0.553, and 0.542, respectively). There was also a statistically significant correlation between the BMI, Ht and FM **(Table 2)**. In regard to skinfolds, only the suprailiac showed a statistical correlation with RMR value. In contrast, all of the body circumferences and breadths showed significant correlation with RMR, especially

Table 1. Descriptive characteristics of examined groups
Tabela 1. Deskriptivne karakteristike ispitivanih grupa

	Aerobic training/*Aerobni trening* N/*br = 21*	Anaerobic training/*Anaerobni trening* N/*br = 21*
Age (years)/*Uzrast (godine)*	22.05±2.67	21.48±1,94
Ht (cm)	182.12±6.06	182.69±7.06
BW (kg)	75.43±8.25	84.52±13.73*
BMI (kg/m²)	22.71±1.86	25.29±3.64*
FM (kg)	6.38±2.64	9.38±7.58
FM (%)	8.26±2.79	10.4±6.41
FFM (kg)	69.06±6.33	75.14±8.95*
SM (kg)	33.36±3.07	37.93±5.08*
SM (%)	44.36±2.32	45.24±4.13
RMR (kcal/24h)	2473±367	2451±429

* p<0,05;
Legend: Ht – height; BW – total body weight; BMI – body mass index; FM – fat mass; FFM – fat-free mass; SM – total skeletal muscle mass; RMR – resting metabolic rate
Legenda: Ht – telesna visina; BW – ukupna telesna masa; BMI – indeks telesne mase; FM – masna masa; FFM – bezmasna masa tela; SM – ukupna skeletna mišićna masa; RMR – energetska potrošnja mirovanja

Table 2. Correlation (Pearson's r coefficient) between the resting metabolic rate and anthropometric parameters in groups engaged in different types of training and its statistical significance
Tabela 2. Korelacija (Pearsonov r koeficijent) energetske potrošnje mirovanja sa antropometrijskim parametrima u grupama na različitom tipu treninga i njena statistička značajnost

Types of training/*Tipovi treninga*	Aerobic/*Aerobni*			Anaerobic/*Anaerobni*			All/*Svi*		
	r		p	r		p	r		p
Ht/*Telesna visina* (cm)	0.163		0.479	0.509	*	0.018	0.361	*	0.019
BW/*Ukupna telesna masa* (kg)	0.360		0.109	0.733	***	< .001	0.542	***	< .001
BMI/*Indeks telesne mase* (kg/m²)	0.361		0.107	0.552	**	0.010	0.425	**	0.005
FM/*Masna masa* (kg)	0.143		0.535	0.443	*	0.044	0.329	*	0.034
FFM/*Bezmasna masa tela* (kg)	0.409		0.066	0.749	***	< .001	0.563	***	< .001
SM/*Ukupna skeletna mišićna masa* (kg)	0.406		0.068	0.789	***	< .001	0.553	***	< .001
Skinfold thicknesses/*Debljina kožnih nabora*									
Chest/*Grudni**	-0.103		0.656	0.302		0.183	0.191		0.225
Subscapular/*Subskapularni**	0.005		0.981	0.386		0.084	0.262		0.094
Midaxillary/*Srednji aksilarni**	0.205		0.373	0.264		0.248	0.207		0.189
Biceps/*Biceps*	0.122		0.597	0.137		0.555	0.113		0.476
Triceps/*Triceps**	-0.152		0.510	0.107		0.645	0.022		0.890
Abdominal/*Abdominalni**	0.055		0.814	0.374		0.095	0.254		0.104
Suprailiac/*Suprailijačni**	0.313		0.167	0.457	*	0.037	0.385	*	0.012
Supraspinale/*Supraspinalni*	0.364		0.105	0.287		0.207	0.278		0.075
Mid-thigh/*Natkolenica**	0.047		0.840	0.230		0.315	0.164		0.299
Medial calf/*Potkolenica*	0.173		0.454	0.253		0.269	0.199		0.206
Σ 7SKF/*Zbir debljina sedam(*) kožnih nabora*	0.094		0.684	0.347		0.124	0.255		0.104
Body circumferences/*Telesni obimi*									
Forearm/*Podlaktica*	0.257		0.262	0.642	**	0.002	0.416	**	0.006
Relaxed upper arm/*Relaksirana nadlaktica*	0.264		0.248	0.658	**	0.001	0.398	**	0.009
Flexed upper arm/*Fleksirana nadlaktica*	0.262		0.251	0.677	***	< .001	0.384	*	0.012
Chest/*Grudi*	0.405		0.068	0.618	**	0.003	0.462	**	0.002
Waist/*Struk*	0.222		0.334	0.609	**	0.003	0.446	**	0.003
Hips/*Kukovi*	0.519	*	0.016	0.415		0.061	0.388	*	0.011
Mid-thigh/*Natkolenica*	0.311		0.169	0.601		0.004	0.459	**	0.002
Calf/*Potkolenica*	0.372		0.097	0.650		0.001	0.498	***	< .001
Corr. arm girth/*Korigovan obim nadlaktice*	0.347		0.123	0.644	**	0.002	0.411	**	0.007
Corr. thigh girth/*Korigovan obim natkolenice*	0.322		0.155	0.549	**	0.010	0.437	**	0.004
Corr. calf girth/*Korigovan obim potkolenice*	0.324		0.151	0.615	**	0.003	0.476	**	0.001
Breadth/*Debljina*									
Femur biepicondylar/*Femura*	0.358		0.111	0.685	***	< .001	0.535	***	< .001
Humerus biepicondylar/*Humerusa*	0.333		0.141	0.404		0.069	0.368	*	0.016

* p < .05, ** p < .01, *** p < .001

the femur biepicondylar breadth and maximum calf girth (r = 0.535 and 0.498, respectively). Scatter plots displaying the correlation between RMR and certain parameters are shown in **Graph 3**.

The measured RMR values in aerobic and anaerobic groups were similar (2472.71 ± 367.14 vs. 2450.9 ± 428.72 kcal/24 h) and there was no statistically significant difference between these two groups (p > 0.05). However, by calculating the relative indices of resting energy expenditure, i.e. dividing the RMR values by total BW, FFM and total SM mass, statistically significant differences were found between the two groups, with significantly higher values in the aerobic group (RMR/BW was 32.98 ± 4.94 vs. 29.15 ± 3.7 kcal/kg/24 h, p = 0.07; RMR/FFM was 35.92 ± 4.98 vs. 32.58 ± 3.85 kcal/kg/24 h, p = 0.02; RMR/SM was 74.37 ± 10.45 vs. 64.63 ± 7.59 kcal/kg/24 h, p = 0.001). **Graph 4** shows differences in relative indices of resting energy expenditure between the two groups of athletes, with indicated level of statistical significance.

Discussion

Exercise on a regular basis is very important for both competitive and recreational athletes. Apart
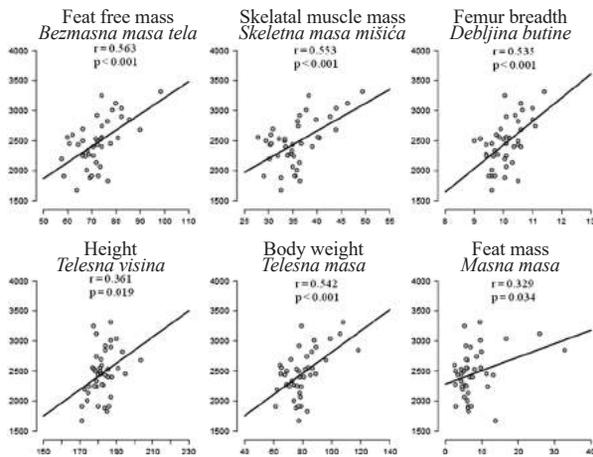
Graph 3. Correlation plots showing the correlation between resting metabolic rate (vertical axis, values in kcal/24 h) and anthropometric parameters (horizontal axis, values in kilograms except height and biepicondylar femur breadth which are expressed in centimeters)
Grafikon 3. *Korelacioni grafikoni sa predstavljenom korelacijom između energetske potrošnje mirovanja (vertikalna osa, vrednosti u kcal/24 h) i antropometrijskih parametara (horizontalna osa, vrednosti u kilogramima, osim visine i debljine femura koji su izraženi u centimetrima)*
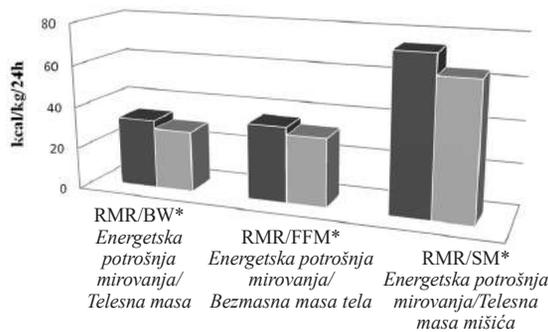
| | RMR/BW* | RMR/FFM* | RMR/SM* |
	Energetska potrošnja mirovanja/Telesna masa	Bezmasna masa tela	Telesna mišićna masa
Aerobic training *Aerobni trening*	32.98	35.92	74.37
Anaerobic training *Anaerobni trening*	29.15	32.58	64.63

Graph 4. Differences in relative indices of metabolic expenditure between two groups (values in kcal/kg/24 h) (* p < 0.05)
Grafikon 4. *Razlike u relativnim pokazateljima metaboličke potrošnje između dve grupe (vrednosti su u kcal/kg/24 h) (* p<0,05)*

from having beneficial effects on a wide array of physiological functions, physical activity also prevents the onset of obesity and its complications, especially in adulthood [29, 30]. The BMI is an indicator that can show if an individual is overweight or underweight, and being easily and widely used for calculation of overweight and obesity, BMI is frequently used as an important tool in health risk assessment. The higher the BMI, the higher is the risk of cardiovascular disease and other associated chronic diseases [31]. Previous studies have shown that BMI is not a good indicator of the amount of fat tissue in athletes, since higher BMI may also indicate increased amount of muscle mass, not necessarily fat [32]. In our study, BMI values were statistically significantly higher in the group engaged in anaerobic training. This result is expected, considering that there was also a significant statistical difference in total BW between the two groups and BMI is a parameter derived from this value.

Our study showed a lower percentage of body fat in both groups of athletes, compared to the average values in the general population. Although this percentage was slightly higher in subjects engaged in anaerobic type of training, this difference was not statistically significant. Optimal values of body fat percentage in our participants range very low and do not differ significantly between groups, so it excludes the influence of this body compartment on RMR variability. Exactly this speaks in favor of FFM in our participants, being one of the main determinants of body weight, and thus also RMR.

Determining the contribution of different body compartments in athletes, i.e. determining their body composition is very important in their profession because the ratio of fat to FFM affects sports achievement. Furthermore, knowing the metabolic parameters, i.e. energy expenditure at rest or during physical activity, completes the physiological profile of each athlete and provides directions for corrections of their training process. Many studies point towards the relationship between anthropometric and metabolic characteristics on one hand, and risk of cardiovascular and metabolic diseases on the other. These are the reasons why assessment and interpretation of anthropometric and metabolic variables in sports medicine should receive special attention [33].

Numerous studies show that engaging in sports leads to an increase in the values of RMR. Such results have been obtained in the studies that are concerned with the impact of aerobic training on RMR values [34], but also in the studies concerning the impact of anaerobic training [35, 36]. Our research revealed somewhat higher values of RMR in athletes engaged in aerobic type of training, but this difference was not statistically significant. It should be noted that subjects in different groups did not have the same amount of FFM, which actually represents the most influential factor determining the RMR of an individual, while the fat mass has a low rate of metabolic activity and thus has a small contribution to RMR [37].

For that reason, in our study, the ratios of resting energy expenditure per kilogram of BW, FFM and SM mass were calculated, then these newly obtained values were compared between two groups and statistically significant differences were obtained. Higher values of these relative indices of metabolic expenditure were obtained in athletes engaged in aerobic type of training and, taking into account that there were no statistically significant

differences in body fat percentages between the groups, it can be concluded that oxygen consumption per kilogram of FFM was higher in the group of athletes engaged in aerobic type of training. Such results can be explained by the fact that aerobic type of training develops the metabolism of an individual in the direction of aerobic capacity, which due to structural differences in basal conditions requires greater amounts of oxygen than anaerobic [20, 21], that is, the SM mass, in which slow-twitch I fibers are found, predominantly has a higher rate of metabolic activity. The absolute amount of SM mass makes the biggest contribution to the FFM compartment and therefore represents the component with the highest influence on RMR [3] which opens opportunities for adequate weight loss training recommendations which are in line with contemporary standpoints on which type of training is more effective against obesity [38].

This research provides potential recommendations about which type of training should be applied in order to increase the RMR and thus, to achieve the desired body weight in the long run. The limitations of this research include a relatively small sample size, young age and lean body composition of the participants, as well as the absence of EPOC measurements. It is necessary to increase the number of subjects with a wider range of age and nutrition. The most reliable data would be gathered if 24-hour energy expenditure was measured in metabolic chambers equipped with different exercise equipment. Then the impact of EPOC in total daily energy expenditure (which surely is not negligible) could be evaluated with a great deal of confidence.

Conclusion

Analyzing the anthropometric parameters of athletes engaged in two different types of training, aerobic and anaerobic, significantly higher values of body weight, body mass index, fat-free mass and skeletal muscle mass were found in subjects engaged in anaerobic type of training (p < 0.05). Although a higher percentage of body fat was measured in these subjects, this difference was not statistically significant. Absolute measured values of resting metabolic rate did not show significant differences between the two groups, but a statistically significant difference was found when energy expenditure values were expressed through relative indices, compared to the total, fat-free and muscle mass with significantly higher values in athletes engaged in aerobic type of training (p < 0.05). Since the resting metabolic rate mostly depends on the fat-free mass, our research speaks in favor of higher rate of metabolic processes in this body compartment in athletes whose training is predominantly aerobic.

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AFFECTIVE STATUS IN CEREBRAL SMALL VESSEL DISEASE

AFEKTIVNI STATUS KOD BOLESTI MALIH KRVNIH SUDOVA MOZGA

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Summary

Introduction. Cerebral small vessel disease is a neurological condition characterized by motor, cognitive and affective disorders, often found on brain magnetic resonance imaging scans in patients with vascular risk factors. Affective disorders may have a major impact on patients' quality of life, although they are often ignored as an entity in cerebrovascular pathology. **Material and Methods.** This prospective study included 80 patients with the diagnosis of cerebral small vessel disease admitted at the Clinic of Neurology, Clinical Center of Serbia in the period from January 1, 2017 to January 1, 2019. Baseline demographic data and brain magnetic resonance findings were obtained along with the results of cognitive function and affective status tests. Data were analyzed using standard statistical tests. **Results. S**tandard screening tests revealed that 51.25% and 33.75% of our patients with cerebral small vessel disease suffer from apathy and depression, respectively. A significant correlation was found between the severity of white matter changes on magnetic resonance scans and apathy (p = 0.0092). Additionally, white matter changes were also significantly associated with depression (p = 0.021). **Conclusion.** Affective disorders are not uncommon in cerebral small vessel disease and apathy was the leading phenomenon among our patients. Since a strong correlation was detected between affective disorders and severity of vascular changes on magnetic resonance scans, we may conclude that both apathy and depression are key features of an underlying brain injury, rather than just comorbidity.

Keywords: Cerebral Small Vessel Diseases; Affective Symptoms; Mood Disorders; Apathy; Depression; Cognition; Magnetic Resonance Imaging; Stroke, Lacunar

Sažetak

Uvod. Bolest malih krvnih sudova mozga je neurološko stanje za koje su karakteristični motorni, kognitivni i afektivni poremećaji i koje se često dijagnostikuje na snimcima mozga magnetnom rezonancijom kod pacijenata sa vaskularnim faktorima rizika. Promene u afektivnom statusu imaju veliki uticaj na kvalitet života pacijenata iako su neretko bile zanemaren fenomen u cerebrovaskularnoj patologiji. **Materijal i metode.** U prospektivnoj studiji obuhvaćeno je 80 pacijenata sa dijagnozom bolest malih krvnih sudova mozga lečenih i ispitivanih na Klinici za neurologiju Kliničkog centra Srbije, u periodu od 1. januara 2017. godine do 1. januara 2019. godine. Analizirani su osnovni demografski podaci, nalaz na magnetnoj rezonanciji mozga i rezultati testova kognitivnih funkcija i afektivnog statusa, upotrebom standardnih statističkih metoda. **Rezultati.** Rezultati testova za apatiju i depresiju ukazali su da je 51,25% bolesnika sa ovom bolešću pokazivalo prisustvo apatije, a 33,75% prisustvo depresivnog poremećaja. Dokazana je statistički značajna povezanost između težine lezija bele mase mozga na magnetnorezonantnim snimcima mozga i apatije (p = 0,0092), odnosno težine lezija bele mase i depresije (p = 0,021). **Zaključak.** Izmene afektivnog statusa nisu retka manifestacija cerebralne bolesti malih krvnih sudova mozga, a najčešći fenomen u našoj grupi bolesnika bila je apatija. Budući da je detektovana značajna povezanost između afektivnih poremaćaja i težine vaskularnih lezija na magnetno-rezonantnim snimcima mozga, smatramo da su apatija i depresivnost najpre deo kliničke slike ove bolesti a ne jedan od komorbiditeta.

Ključne reči: bolest malih krvnih sudova; afektivni simptomi; poremećaji raspoloženja; apatija; depresija; kognicija; magnetna rezonanca; lakunarni moždani udar

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Introduction

Cerebral small vessel disease (CSVD) is a progressive vascular disease affecting small perforating arteries, arterioles, capillaries and venules of the brain, characterized by typical clinical and neuroimaging findings [1]. Magnetic resonance imaging (MRI) of the brain shows lacunar infarctions, white-matter hyperintensities (WMH) and cerebral microbleeds along with cortical atrophy and enlargement of perivascular spaces [2]. Development and progression of CSVD is associated with common vascular risk factors, leading to the highest prevalence of CSVD among elderly patients [3]. Besides motor (pyramidal and extrapyramidal signs, gait difficulties) and sensory manifestations, clinical presentation of CSVD also includes loss of sphincter control and changes in cognitive function and affective status.

Cognitive decline in CSVD varies from mild vascular-cognitive impairment (VCI) to overt vascular

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dementia with main affection of frontal and subcortical cerebral areas [1]. Changes in affective status, including depression and apathy, are not uncommon but are likely underdiagnosed and undertreated [1]. It can be challenging to differentiate depression and apathy as they share many overlapping symptoms. Apathy is characterized by the loss of motivation, pathological indifference and absence of common emotional reactions, but on the other hand, loss of motivation is also one of the major symptoms of depression [4, 5]. Also, patients are at an increased risk of depressive episode after an ischemic stroke of any type, and 20 – 25% of all strokes are due to CSVD. Both apathy and depression are associated with a decline in quality of life, failure of the rehabilitation process and an increased risk of cognitive impairment [1, 5, 6].

Full neuropsychological and psychiatric assessment of patients with CSVD is time-consuming and expensive, but bedside screening tests may fail to differentiate apathy from depression. It is possible to overestimate the presence of depression in patients with CSVD, and at same time underestimate the number of cases with apathy. This difference could be clinically significant, because common depression treatment strategies may not target symptoms of apathy [7]. The aim of this study was to analyze affective status in a cohort of inpatients treated for symptomatic CSVD using standard screening tests.

Material and Methods

This prospective study was approved by the Ethics Committee of the Clinical Center of Serbia and included 80 patients with clinical an MR diagnosis of CSVD, admitted to the Clinic of Neurology, University of Belgrade, in the period January 1, 2017 - January 1, 2019. Baseline demographic data, risk factors and clinical presentations were collected from medical records, and all participants underwent cognitive function and affective status testing as well as standard MR scanning. All patients were diagnosed with CSVD, based on medical history, neurological examination, MR findings and results of additional tests used to exclude similar neurological conditions.

Patients included in this study completed standardized screening questionnaires on their symptoms of depression, apathy and anxiety [8–10]. Patients were asked to fill out two more questionnaires concerning their quality of life and presence of major stressful life events [11, 12]. All patients were tested for their cognitive status using standardized methods [13].

The presence and severity of depression symptoms was evaluated by the second revision of Beck Depression Inventory (BDI-II) test. This self-report inventory consists of 21 multiple-choice questions with a maximum total score of 63 points. Patients with a total score of 14 points or more, were classified as positive for symptoms of depression, while those with a total score less than 14 points were classified as depression free [10]. The presence and severity of apathy symptoms was evaluated by using a modified version of Apathy Evaluation Scale (AES). This self-report inventory consists of 14 multiple-choice questions with a maximum total score of 42 points. Patients with a total score of 14 points or more were classified as apathy-positive group, while those with a total score less than 14 points were classified as apathy-free [8]. The presence and severity of anxiety symptoms was evaluated by Beck Anxiety Inventory (BAI). This self-report inventory consists of 21 multiple-choice questions with a maximum total score of 63 points. Patients with a total score of 22 points or more were classified as anxiety-positive, while those with a total score of 21 points or less were classified as anxiety-free [9].

The quality of life was assessed using the Serbian translation of a 36-Item Short Form Health Survey (SF-36), taking into consideration only the total scores [11]. Experience of stressful life events was registered with the Holmes-Rahe Stress Inventory (HRSI). This self-report inventory consists of 43 different major stressful life events. Patients were asked to identify if such events happened within the last year. According to the total result, we made two groups of patients: experiencing mild stress (150 points or less) and experiencing excessive level of stress (150 points or more) [12].

Mini-Mental State Examination (MMSE) test was used for the detection and measurement of cognitive impairment. Patients with a total score of 24 points or more were classified as patients with normal cognition, while those with a total score less than 24 points were classified as cognitively impaired [13].

The analysis included baseline demographic data on sex and age, as well as results of neuropsychological tests and MR lesion severity. Brain MR findings were analyzed for the presence of lacunar infarctions, white matter hyperintensities (WMH) and Age-Related White Matter Changes Scale (ARWMC) and Fazekas scale. The ARWMC scale illustrates overall cerebral damage with WMH and lacunar infarctions, with a range from 0 (no damage) to 30 (maximal damage) [14]. Fazekas scale is used to quantify the amount of WMHs seen on brain MR scans. All brain MR scans are given a grade ranging from 0 (absent lesions) to grade 4 (large confluent areas), with separate scoring for periventricular (PV) and subcortical (SC) lesions [15].

All neuropsychological testing and MR grading were performed by trained examiners blinded for clinical data. Data were processed using IBM SPSS 23 program (IBM, New York, USA). We used de-

scriptive and analytical statistical methods. Numerical variables were calculated in terms of arithmetic mean and standard deviation. To determine a significant difference and/or correlation between the parameters, Chi-squared test and Spearman test of correlation were used. The $p < 0,05$ was considered statistically significant.

Results

Demographic data
Our study included a total of 80 participants diagnosed with CSVD, 58 (72.5%) female and 22 (27.5%) male patients, mean age of 56.3 ± 15.0 years, ranging from 23 to 81 years.

Affective status
Results of neuropsychological testing are shown in **Table 1**. Symptoms of apathy were recorded in more than half of our patients (41 patients or 51.25%). One third (27 or 33.75%) of patients reported symptoms of depression. Symptoms of anxiety were less common, present only in 12 patients (15%) **(Table 1)**.

Brain MR findings
Brain MR findings are summarized in **Table 2**. MR detected lacunar infarctions in all participants (100.0%), while presence of WHM was less frequent (37.5%). The mean value of ARWMC score was relatively low (8.25, median 6). Fazekas grading scales indicated presence of typical PV lesions in 27 (33.75%) patients, while SC lesions were present in 79 (98.75%) patients. The mean value of both PV and SC WMH scores on Fazekas scale was 1 (mild lesions).

Correlation between neuropsychological scores and demographic data
Male patients (77.3%) reported symptoms of apathy more often than female participants (41.4%, p = 0.0056), and also had higher total scores on corresponding inventory (mean score 16.8 in men and 12.6 in women; p = 0.011) **(Table 3)**.
No significant difference was found between the sexes regarding depressive symptoms (40.91% in males and 31.03% in female patients; p = 0.426). The same findings were detected for symptoms of anxiety (18.18% in male and 13.79% in female patients; p = 0.750). The mean scores on applied tests also did not differ between sexes **(Table 3)**.

Correlation between affective status and cognitive changes
A significant correlation was found between presence of apathy and VCI (r = 0.309, p = 0.00528). No such correlation was found in case of anxiety (r = -0.0737, p = 0.518) but it was at the level of statistical trend for depression (r = 0.206, p = 0.067).

Correlation of affective status and quality of life
Presence of apathy (r = -0.448, p < 0.001), depression (r = -0.410, p = 0.001) as well as anxiety (r =

Table 1. Frequency of affective disorders and mean scores on screening inventories in patients with CSVD
Tabela 1. *Učestalost afektivnih poremećaja i srednje vrednosti rezultata skrining testova u grupi ispitanika sa BMKS*

	Screening outcome/*Ishod skrininga*	N/*Broj*	%	x̄+SD
Depression/*Depresija*	Present/*Prisutna*	27	33.75	21.00 + 7.19
	Not present/*Nije prisutna*	53	66.25	4.47 ± 3.50
Anxiety/*Anskioznost*	Present/*Prisutna*	12	15.0	32.08 ± 4.79
	Not present/*Nije prisutna*	68	85.0	8.63 ± 5.27
Apathy/*Apatija*	Present/*Prisutna*	41	51.25	18.66 + 4.42
	Not present/*Nije prisutna*	39	48.75	8.62 + 3.11

Legenda: BMKS – bolest malih krvnih sudova

Table 2. Severity of vascular lesions detected on brain MR
Tabela 2. *Težina vaskularnih lezija na magnetno-rezonantnim snimcima mozga*

	Number of patients/*Broj pacijenata* (%) x̄ + SD
Lacunar infarctions, n (%)/*Lakunarni infarkti*	80 (100.0)
WMH/HBM, n (%)	30 (37.5)
ARWMC, x̄ + SD (median)	8.25 + 5.17 (6.0)
Fazekas PV, x̄ ± SD (mod)	0.76+ 1.13 (0)
Fazekas SC, x̄ ± SD (mod)	1.39 + 0.61 (1)

Legend: WMH - white matter hyperintensities, ARWMC - Age Related White Matter Changes, PV periventricular, SC - subcortical, ARWMC – Age Related White Matter Changes score
Legenda: HBM – hiperintenziteti bele mase, PV – periventrikularno, SC – supkortikalno, ARWMC – skor lezija bele moždane mase u vezi sa starošću

Table 3. Achievements on screening inventories between sexes
***Tabela 3.** Razlike u rezultatima skrining testova među polovima*

	Female/*Žene*	Male/*Muškarci*	p/*p*
Depression, x̄+SD/*Depresija*	9.53 + 9.53	11.41+8.86	0.426
Anxiety, x̄±SD/*Anksioznost*	11.93 + 10.18	12.73 + 9.28	0.750
Apathy, x̄+SD/*Apatija*	12.62 + 6.01	16.77 + 6.28	0.011
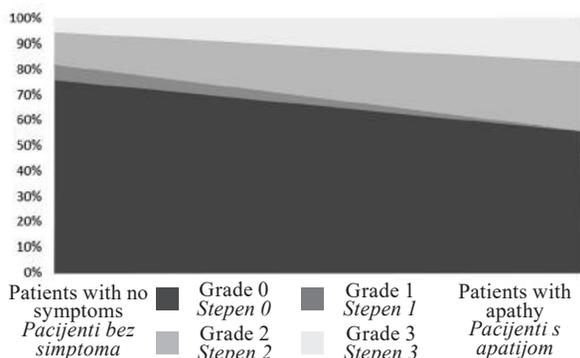
Graph 1. Correlation of periventricular white-matter lesions expressed in Fazekas grading scale and presence of apathy in patients with CSVD
***Grafikon 1.** Povezanost periventrikularnih lezija bele mase izraženih u Fazekas gradusima i prisustva apatije kod pacijenata sa bolestima malih krvnih sudova mozga*

Graph 2. Correlation of periventricular white-matter lesions expressed in Fazekas grading scale and presence of depression in patients with CSVD
***Grafikon 2.** Povezanost periventriknlarnih lezija bele mase izraženih u Fazekas gradnsima i prisustva depresije kod pacijenata sa BMKS mozga*

-0.378, p = 0.002) in our patients was associated with lower quality of life according to total scores on SF-36 inventory.

Correlation of affective status and major stressful life events

No significant correlation was found between reporting major stressful life events and outcomes on depression and anxiety inventories (p for both > 0.1), but it approached statistical significance for apathy (r = 0.205, p = 0.091).

Correlation between affective and cognitive status and brain magnetic resonance imaging findings

A significant correlation was found between AR-WMC scores and results on BDI (p = 0.0092). There was also a significant correlation between ARWMC scores and results on AES (p = 0.021). On the other hand, no correlation was found between ARWMC scores and results on BAI (p = 0.368) **(Table 4)**.

The strongest association was found between PV scores on Fazekas scale and presence of apathy (p = 0.048) as well as depression symptoms (p = 0,015; **Graphs 1** and **2**). Other correlations were negative, except for the apathy symptoms and Fazekas SC scores, which approached statistical significance (p = 0.081).

A statistically strong association was found for performance on cognitive test (MMSE) and Fazekas PV and SC scores, respectively (p < 0.001).

Discussion

Our study results indicate that changes in affective status are not uncommon in patients with CSVD, surprisingly leading with apathy symptoms. More

Table 4. Relationship between affective symptoms and severity of vascular lesions on MR
***Tabela 4.** Odnos afektivnog statusa i težine vaskulamih lezija mozga na magnetno-rezonantnim snimcima*

	Screening outcome/*Ishod testa*	ARWMC score, x̄ + SD/*ARWMC skor, x̄ ± SD*	p/*p*
Depression/*Depresija*	Present/*Prisutna*	10.33 + 6.09	0.0092
	Not present/*Nije prisutna*	7.19 + 4.32	
Anxiety/*Anksioznost*	Present/*Prisutna*	9.50 + 5.30	0.368
	Not present/*Nije prisutna*	8.03 + 5.16	
Apathy/*Apatija*	Present/*Prisutna*	9.54 + 5.84	0.021
	Not present/*Nije prisutna*	6.89 + 4.00	

Legend: ARWMC – Age Related White Matter Changes score
Legenda: ARWMC – skor lezija bele moždane mase u vezi sa starošću

than a half of patients included in this study (51.25%) reported symptoms of apathy, while depression, typically recognized as a CSVD manifestation, was leading with 33.75%. Current research on affective status in patients with CSVD also came to similar conclusions, revealing that in some cohorts of patients apathy was the leading symptom in early-stage disease [5, 7].

Both apathy and depression were associated with cognitive decline and decrease in quality of life of patients with CSVD, especially in those with dysexecutive syndrome which is one of the major manifestations of the disease [4, 5]. Our study results are in accordance with this finding, showing a significant change in affective status as well as cognitive impairment and a decline in quality of life.

In accordance to Alexopoulos vascular depression hypothesis, underlying pathological mechanism could be a syndrome of disconnection due to vascular white matter lesions which affect frontolimbic and frontostriatal networks [15, 16]. Results of self-report screening inventories for apathy and depression have shown excellent correlation with vascular lesions predominantly in white matter, quantified by ARWMC and Fazekas scales. The research of Hollock et al. reported that a more intense association exists between white matter lesions and apathy due to disruption of cortico-subcortical pathways [5, 7]. Symptoms of apathy are linked to lesions of limbic associative pathways such as anterior cingulate cortex, fornix and fasciculus uncinatus [5, 15]. Furthermore, presence of depression relates to higher ARWMC scores. However, it is a composite score, so exact localization of the lesions was not analyzed in our study. In accordance to current research, our study results reveal significant association between PV lesions and changes in affective status, whereas SC lesions were more frequently linked to cognitive decline [5, 15].

These findings are valuable because they could enable early identification of patient neuroradiological profile thus creating different clinical subgroups more susceptible to cognitive and/or affective decline etc.

We present data from well-defined cohort of patients with CSVD who underwent comprehensive clinical, neuropsychological and neuroimaging assessment. Limitations of our study are related to nature of self-report testing, a method available only to patients with mild motor and/or cognitive impairment, and therefore more severely affected patients were not included in the study. Nevertheless, these short screening inventories are very accessible, so their use in everyday practice is simple, affordable and achievable in hospital conditions for early detection on affective status decline. Also, our study has not considered the impact of different therapeutics frequently used in patients with neuropsychiatric disorders such as antidepressants and tranquilizers, which could possibly interfere with the results [16]. Since vascular depression and apathy are often resistant to available pharmacotherapy, prevention of vascular lesions which could be the underlying cause of these phenomena and further research on new therapeutic strategies is of utmost importance [17].

Conclusion

Results of our study indicate that changes in affective status are not uncommon in patients with cerebral small vessel disease. These changes correlate with cognitive decline and severity of vascular lesions detected on brain magnetic resonance imaging. Since these changes are undoubtedly linked to decrease in quality of life, early recognition of these phenomena is of utmost importance for selecting the best therapeutic strategies.

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PREDICTION OF CARDIOVASCULAR RISK AMONG MALE PATIENTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS IN VOJVODINA, SERBIA – A SINGLE CENTRE STUDY

PROCENA KARDIOVASKULARNOG RIZIKA KOD MUŠKARACA INFICIRANIH VIRUSOM HUMANE IMUNODEFICIJENCIJE U VOJVODINI, SRBIJA – MONOCENTRIČNA STUDIJA

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Summary

Introduction. Human immunodeficiency virus infection is a disease of the modern era and it is estimated that there are more than 30 million infected individuals worldwide. Although the major cause is still unclear, patients infected with human immunodeficiency virus are at higher risk of ardiovascular diseases by 61% compared to general population. **Material and Methods**. This study included 111 male patients infected with human immunodeficiency virus treated at the Clinic of Infectious Diseases, Novi Sad, Serbia from January 2008 to December 2018. Five cardiovascular risk scores were used: Data Collection on Adverse Events of Anti-human immunodeficiency virus Drugs, Framingham 10-year Heart Score, Framingham 5-year Heart Score, prediction algorithm for cardiovascular disease and atherosclerotic cardiovascular disease risk estimator, at the beginning of the treatment, whereas cardiovascular events were recorded during the following 10 years. **Results.** Data Collection on Adverse Events of Anti-human immunodeficiency virus Drugs, Framingham 10-year Heart Score, Framingham 5-year Heart Score, and prediction algorithm for cardiovascular disease are tools that can identify individuals infected with human immunodeficiency virus at cardiovascular risk with statistical significance. The prediction algorithm for cardiovascular disease provides superior risk estimation compared to other scores. The atherosclerotic cardiovascular disease risk estimator did not show to be a marker of cardiovascular risk prediction among this population of patients. **Conlusion.** The above mentioned cardiovascular risk prediction algorithms, developed for general population, and Data Collection on Adverse Events of Anti-human immunodeficiency virus Drugs score, specific for population infected with human immunodeficiency virus, allow accurate cardiovascular risk estimation. Until the development of more specific algorithms, these scores are adequate tools for identification of patients at risk, providing prevention measures and treatment of cardiovascular disease.
Key words: HIV Infections; Vulnerable Populations; Cardiovascular Diseases; Risk Assessment; Algorithms; ROC Curve; Sensitivity and Specificity; Correlation of Data

Sažetak

Uvod. Infekcija virusom humane imunodeficijencije je bolest modernog doba koja obuhvata više od 30 miliona inficiranih u svetu. Iako je razlog još uvek nejasan, učestalost kardiovaskularnih bolesti među ovom populacijom je veća za 61% u odnosu na opštu populaciju. **Materijal i metode.** Ova studija je uključila 111 muškaraca inficiranih virusom humane imunodeficijencije, lečenih na Klinici za infektivne bolesti Kliničkog centra Vojvodine, od januara 2008. do decembra 2018. godine. Pet skorova za predviđanje kardiovaskularnog rizika: prikupljanje podataka o štetnim efektima lekova prostiv virusa humane imunodeficijencije, desetogodišnji rizik za razvoj kardiovaskularnih bolesti, petogodišnji rizik za razvoj kardiovaskularnih bolesti, treća verzija algoritma za procenu rizika za razvoj kardiovaskularnih bolesti i procena rizika za razvoj aterosklerotskih kardiovaskularnih bolesti su izračunati na početku tretmana, a pojava kardiovaskularnih događaja je praćena tokom narednih 10 godina. **Rezultati.** Prikupljanje podataka o štetnim efektima lekova protiv virusa humane imunodeficijencije, desetogodišnji rizik za razvoj kardiovaskularnih bolesti, petogodišnji rizik za razvoj kardiovaskularnih bolesti i treća verzija algoritma za procenu rizika za razvoj kardiovaskularnih bolesti statistički značajno predviđaju kardiovaskularni rizik među muškarcima pozitivnim na virus humane imunodeficijencije. Treća verzija algoritma za procenu rizika za razvoj kardiovaskularnih bolesti je superiornija u predviđanju kardiovaskularnog rizika u poređenju sa drugim skorovima. Skor za procenu rizika za razvoj aterosklerotskih kardiovaskularnih bolesti nije se pokazao kao odgovarajući za korišćenje u predviđanju kardiovaskularnog rizika kod ove grupe pacijenata. **Zaključak.** Pomenuti skorovi za predviđanje kardiovaskularnog rizika koji se upotrebljavaju u opštoj populaciji i skor treće verzija algoritma za procenu rizika za razvoj kardiovaskularnih bolest specifičan za populaciju inficiranu virusom humane imunodeficijencije mogu da se koriste za procenu rizika kod pacijenata pozitivnih na virus humane imunodeficijencije. Do formiranja specifičnijih algoritama, ovi skorovi mogu adekvatno da ukažu na pacijente pod rizikom i time pomognu kod tretmana i prevencije kardiovaskularnih bolesti.
Ključne reči: HIV infekcije; ranjive populacije; kardiovaskularna oboljenja; procena rizika; algoritam; ROC kriva; senzitivnost i specifičnost; korelacija

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Introduction

Human immunodeficiency virus (HIV) infection is a disease of the modern age and according to some estimates there are more than 30 million infected individuals worldwide. Although prolonged life expectancy is mostly due to antiretroviral therapy, the virus or antiretroviral therapy can lead to higher percentage of chronic diseases or comorbidities in this population, especially cardiovascular diseases CVDs [1].

Studies have shown that the risk of CVDs is by 61% higher in HIV-infected individuals, although the major cause is still unclear [2]. The underlying pathogenesis is a complex combination of traditional risk factors, viral infection and antiretroviral therapy [3].

While there is a clear contribution of traditional risk factors and their high prevalence in people living with HIV, the infection itself causes chronic inflammations and metabolic disturbances caused by antiretroviral therapy that should not be underestimated [4].

There is no doubt that adequate cardiovascular risk calculation may contribute to better management and prevention of morbidity and mortality among this population, but clinicians are still searching for the most suitable risk score [5].

The Framingham heart score (FHS) is widely used to estimate the risk of CVDs in general population and includes general risk factors such as gender, age, systolic blood pressure (SBP) and antihypertensive treatment, high density lipoprotein (HDL), total cholesterol, and the smoking status [6, 7]. Although FHS is recommended in HIV population, there is a dispute among studies, with some pointing to underestimation of cardiovascular risk using this scoring system [5, 7].

American society of cardiology and American heart association (ASC/AHA) developed an atherosclerotic cardiovascular disease (ASCVD) risk estimator which determines 10-year risk of CVD and stroke. It was created for general population aged 40 – 79 years, and includes the following variables: age, gender, race, total and HDL cholesterol, systolic and diastolic blood pressure, antihypertensive therapy, diabetes, and smoking status [8]. Some cohorts have shown that it underestimates the CVD risk, among HIV-infected individuals compared to FHS [9].

The Data Collection on Adverse Events of Anti-HIV Drugs (DAAD) CVD risk score is the only one developed for cardiovascular risk stratification in this specific population and besides traditional risk factors also includes variables such as T-helper (CD4) cell count and antiretroviral therapy exposure [9].

The prediction algorithm for cardiovascular disease (QRISK) score was first established in 2007 in England, and since then it has been updated every year [10]. The QRISK score 3 is the latest version and includes both traditional risk factors (age, sex, ethnicity, cholesterol values, systolic blood pressure and antihypertensive treatment, smoking, diabetes, angina in a first-degree relative) and plenty of variables which are not included in other risk scores, such as atrial fibrillation, chronic kidney disease (stage 3, 4, or 5), migraine, corticosteroid therapy, systemic lupus erythematosus (SLE), atypical antipsychotics, severe mental illness, and erectile dysfunction in men [11]. The estimation of CVD risk by QRISK score has been poorly investigated in HIV-infected individuals [12].

Considering the importance of preventive measures, and adequate screening for those at risk for CVD, the aim of this study was to assess which scoring system is the most accurate for cardiovascular risk evaluation in HIV-infected individuals.

Material and Methods

This retrospective study included 111 HIV-infected male patients treated at the Clinic of Infectious Diseases, Clinical Center of Vojvodina, Novi Sad, Serbia in the period from January 2008 to December 2018. Data were collected from hospital database, using parameters gathered on the time of setting the HIV infection diagnosis in 2008, and medical records on the onset of cardiovascular events (myocardial infarction, stroke or death caused by CVD).

Five cardiovascular risk scores were calculated at the beginning of the treatment, using online available certified calculators: FHS 5-year risk, FHS 10-year risk (https://chip.dk/Tools-Standards/Clinical-risk-scores), ASCVD score (http://www.cvriskcalculator.com/), DAAD score (https://chip.

Table 1. Number of patients included in score calculation
Tabela 1. Broj pacijenata uključenih u računanje skora

Score/*Skor*	FHS 10	QRISK3	FHS 5	DAAD	ASCVD
Number of patients included/*Broj uključenih pacijenata*	75	108	75	106	39

Legend/*Legenda*: FHS 10 – Framingham heart score/*desetogodišnji rizik za razvoj kardiovaskularnih bolesti,* FHS5 – Framingham heart score/*petogodišnji rizik za razvoj kardiovaskularnih bolesti,* QRISK3 – Prediction algorithm for cardiovascular disease 3/*treća verzija algoritma za procenu rizika za razvoj kardiovaskularnih bolesti,* DAAD – Data Collection on Adverse Effects of Anti-HIV Drugs Study/*Prikupljanje podataka o štetnim efektima lekova protiv virusa humane imunodeficijencije,* ASCVD – Atherosclerotic Cardiovascular Disease risk estimator/*Procena rizika za razvoj aterosklerotskih kardiovaskularnih bolesti*

Table 2. Differences between FHS 10 risk categories
Tabela 2. *Razlika između kategorija rizika kod FHS 10*

FHS 10		Cardiovascular events/*Kardiovaskularni incidenti*			
		No/*Ne*		Yes/*Da*	
		N/*Broj*	Row/*Red* N %	N	Row/*Red* N %
Risk level *Kategorija rizika*	Low/*Nizak*	36	94.7%	2	5.3%
	Medium/*Srednji*	18	78.3%	5	21.7%
	High/*Visok*	7	50%	7	50%

Legend/*Legenda*: FHS 10 – Framingham heart score/*Desetogodišnji rizik za razvoj kardiovaskularnih bolesti*

Table 3. Sensitivity and specificity of each score
Tabela 3. *Senzitivnost i specifičnost skorova*

Score/*Skor*	FHS 10	FHS 5	QRISK 3	DAAD
AUC (95%CI)	0.83 (0.71-0.94)	0.77 (0.62-0.91)	0.92 (0.87-0.97)	0.77 (0.65-0.92)
Sensitivity/*Senzitivnost* %	81,3	73,3	93,8	80
Specificity/*Specifičnost* %	75	76,3	83,3	71,3

Legend/*Legenda*: FHS 10 – Framingham heart score/*desetogodišnji rizik za razvoj kardiovaskularnih bolesti;* FHS5 – Framingham heart score/*petogodišnji rizik za razvoj kardiovaskularnih bolesti;* QRISK3 – Prediction algorithm for cardiovascular disease 3/*treća verzija algoritma za procenu rizika za razvoj kardiovaskularnih bolesti;* DAAD – Data Collection on Adverse Effects of Anti-human immunodeficiency virus Drugs Study/*Prikupljanje podataka o štetnim efektima lekova protiv virusa humane imunodeficijencije,* AUC – Area under the curve/*Površina ispod krive*

Table 4. Correlation coefficient among scores
Tabela 4. *Koeficijent korelacija između skorova*

			ASCVD	QRISK3	FHS 10	FHS 5	DAAD
Spearman's Rho *Spirmanov ro*	ASCVD	Correlation Coefficient/*Koeficijent korelacije*	1	.774[**]	.696[**]	.828[**]	.739[**]
		Sig. (2-tailed)/*Značajnost (2-zavisna uzorka)*	.	0	0	0	0
		N/*Broj*	39	39	39	38	38
	QRISK3	Correlation Coefficient/*Koeficijent korelacije*	.774[**]	1	.901[**]	.919[**]	.892[**]
		Sig. (2-tailed)/*Značajnost (2-zavisna uzorka)*	0	.	0	0	0
		N/*Broj*	39	112	112	74	106
	FHS 10	Correlation Coefficient/*Koeficijent korelacije*	.696[**]	.901[**]	1	.964[**]	.892[**]
		Sig. (2-tailed)/*Značajnost (2-zavisna uzorka)*	0	0	.	0	0
		N/*Broj*	39	112	112	74	106
	FHS 5	Correlation Coefficient/*Koeficijent korelacije*	.828[**]	.919[**]	.964[**]	1	.893[**]
		Sig. (2-tailed)/*Značajnost (2-zavisna uzorka)*	0	0	0	.	0
		N/*Broj*	38	74	74	74	74
	DAAD	Correlation Coefficient/*Koeficijent korelacije*	.739[**]	.892[**]	.892[**]	.893[**]	1
		Sig. (2-tailed)/*Značajnost (2-zavisna uzorka)*	0	0	0	0	.
		N/*Broj*	38	106	106	74	106

Legend/*Legenda*: FHS 10 – Framingham heart score/*desetogodišnji rizik za razvoj kardiovaskularnih bolesti;* FHS 5 – Framingham heart score/*petogodišnji rizik za razvoj kardiovaskularnih bolesti,* QRISK3 – Prediction algorithm for cardiovascular disease 3/*treća verzija algoritma za procenu rizika za razvoj kardiovaskularnih bolesti,* DAAD – Data Collection on Adverse Effects of Anti-human immunodeficiency virus Drugs Study/*Prikupljanje podataka o štetnim efektima lekova protiv virusa humane imunodeficijencije,* ASCVD – Atherosclerotic Cardiovascular Disease risk estimator/*Procena rizika za razvoj aterosklerotskih kardiovaskularnih bolesti*

dk/Tools-Standards/Clinical-risk-scores), and QRISK3 score (https://qrisk.org/three/).

Three patients with missing data were excluded from the study. FHS 5 and FHS 10 cardiovascular risk estimation included patients who were more than 30 years old. ASCVD included patients over 40 years of age at the beginning of the treatment. QRISK 3 was calculated for 108 patients. All statistical analyses were performed using SPSS version 21.

The receiver operating characteristic (ROC) curve was used to determine the optimal cut-off value of each score for the prediction of CVDs. The area under the curve (AUC), and 95% confidential interval, sensitivity, and specificity were reported for the whole

sample. The ROC curves were interpreted as the probability that the estimated interval values can adequately discriminate patients with cardiovascular risk and without it (where 0.5 is chance discrimination and 1.0 is perfect discrimination). The correlation coefficient was calculated between the tests.

Results

A total of 111 HIV-positive male patients were included in this study, with an average age of 37.48 ± 11.67 years, at the time when HIV infection was diagnosed. All the scores except ASCVD could estimate the cardiovascular risk among HIV-infected individuals with statistical significance. All patients included in calculation are presented in **Table 1**. Cut off values for each score were calculated using ROC curve.

The prediction algoritam for cardiovascular disease (QRISK) score of 4.35 or higher identified cardiovascular risk in HIV-infected individuals with sensitivity of 93.8%, specificity of 83.3% (AUC 0.92, 95% CI, 0.87 – 0.97, p < 0.000).

In risk assessment, the value of 10 or higher calculated using FHS 10-year showed a sensitivity of 81.3% and specificity of 75% (AUC 0.83, 95% CI, 0.71 – 0.94, p < 0.000).

There was a difference in frequency of cardiovascular events among FHS 10-year risk categories (χ2 = 13,693; p = 0,001). Patients with high risk scores, compared to low category risk scores, showed higher incidence of cardiovascular events (7 (50.0%) vs. 2 (5.3%); p < 0,001)) **(Table 2)**.

Framingham 5-year heart score of 2.75 (AUC 0.77, 95% CI, 0.62 - 0.91, p < 0.001) and DAAD of 2.32 (AUC 0.77, 95% CI, 0.65 - 0.92, p < 0.000) showed a sensitivity of 73.3%, 80% and specificity of 76.3%, 71.3% in cardiovascular risk assessment, respectively **(Table 3)**. All tests showed good correlation coefficients between each other **(Table 4).**

Discussion

This study investigated 4 scoring algorithms developed for general population and 1 developed specifically for HIV population to assess the cardiovascular risk.

In our country, CVDs are number one cause of death since 1980s, and in 2007 the CVD-related deaths in all-cause mortality accounted for 56% [13].

The trend of rising CVD mortality in HIV-infected individuals is growing in importance (14). In 2010, 19% of HIV-infected individuals had at least one CVD [15, 16].

While there are many data showing HIV infection itself seems to be one of the risk factors for CVD, there are no such algorithms to include HIV infection as a parameter [17, 18].

Our study has shown that FHS 5 and FHS 10-year scores, DAAD score and QRISK3 score are reasonable to use for CVD risk estimation among HIV-infected individuals.

However, ASCVD score did not predict the risk accurately, which was also reported in some previous studies [5]. In our study this can be explained by a small sample size, while only patients who were 40 years or older were included in the score calculation.

In our study, QRISK3 showed the highest accuracy in identifying subjects at risk of CVDs, with the highest sensitivity and specificity compared to other scores. The score itself was developed for risk stratification of the population of the United Kingdom, while there are no clear limits of using it for CVD risk estimation in other populations [11]. The role of this algorithm and its accuracy for HIV-infected individuals has not been investigated in previous studies. It will be interesting to check the sensitivity of this score in a larger group of HIV patients.

A recent study conducted in the Netherlands found that FHS risk score is acceptable in HIV-infected individuals, as was confirmed by our results. Both FHS scores, which estimate the 5 and 10-year CVD risks, were good at risk stratification in our study [19].

Data Collection on Adverse Events of Anti-HIV Drugs is the only scoring algorithm developed for HIV patients, and includes antiretroviral therapy exposure and duration of the treatment. The role of this score in CVD risk assessment is still unclear [5, 19, 20]. Its property to identify subjects at risk is inferior compared to QRISK3 score, but also statistically significant. This implicates that in our study the sample size or other parameters, rather than antiretroviral therapy exposure or duration of the treatment, contribute to CVD risk. However, we cannot underestimate the role of some drugs, for example abacavir and duration of exposure, as well as the duration of HIV infection prior to the treatment [21, 22].

All scores showed high correlation coefficient between each other which shows that one score calculation is enough to determine the cardiovascular risk.

The main advantage of this study is that it estimated the cardiovascular risk among HIV-infected subjects in Vojvodina for the first time, but the sample size is relatively small.

Conclusion

This study showed that four risk scores (Data Collection on Adverse Events of Anti-human immunodeficiency virus Drugs, Framingham 10-year Heart Score, Framingham 5-year Heart Score, prediction algorithm for cardiovascular disease) are reasonable to use for cardiovascular risk stratification among individuals infected with human immunodeficiency virus, while the atherosclerotic cardiovascular disease risk estimator is not suitable for this specific population. The prediction algorithm for cardiovascular disease 3 score showed the best results in identification of subjects at risk, with high sensitivity and specificity, while its usage among subjects infected with human immunodefi-

ciency virus has not been previously investigated. This opens a topic for future investigations. Although cardiovascular risk stratification among individuals infected with human immunodeficiency virus remains challenging, studies including new parameters in scoring algorithms may provide additional insights. Until then, the usage of algorithms developed for general population is useful for identification of patients at risk.

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SUPPLEMENTATION WITH THREE MACULAR CAROTENOIDS IN PATIENTS WITH EARLY STAGE AGE-RELATED MACULAR DEGENERATION

SUPLEMENTACIJA UPOTREBOM TRIMA MAKULARNA KAROTENOIDA KOD PACIJENATA SA RANIM STADIJUMOM SENILNE DEGENERACIJE MAKULE

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Summary

Introduction. Macula lutea is the central part of the retina and it houses carotenoid macular pigments – lutein, zeaxanthin, and meso-zeaxanthin. It is considered that carotenoids can prevent or slow down the progression of the early age-related macular degeneration in some patients. Therefore, the aim of this study was to examine the effects of supplementation with all three macular pigments on vision quality in patients with early age-related macular degeneration. **Material and Methods.** This prospective study included 15 patients (25 eyes in total) with early age-related macular degeneration. All visual tests - visual acuity, glare sensation and contrast sensitivity, were performed at baseline and after 6 months of continuous supplementation with 10 mg lutein, 2 mg zeaxanthin, and 10 mg meso-zeaxanthin. **Results.** The results showed that glare sensitivity improved in 20% of cases. Most of the examined eyes (76%) presented without visual acuity deterioration. Similar results were obtained for the contrast sensitivity test, where in 80% of cases vision quality remained stable or improved. There were no statistically significant differences between average values of examined parameters at baseline and after 6 months of supplementation. **Conclusion.** In most of the examined eyes (over 75%) visual quality remained stable or improvement of measured parameters was observed. This type of supplementation may improve visual performance or its preservation in patients with early age-related macular degeneration.
Key words: Macular Degeneration; Visual Acuity; Dietary Supplements; Macular Pigment; beta Carotene; Lutein; Zeaxanthins; Risk Factors

Sažetak

Uvod. Makula lutea – žuta mrlja predstavlja centralni deo retine u kojoj se akumulira makularni pigment sačinjen od karotenoida – luteina, zeaksantina i mezozeaksantina. Smatra se da ova jedinjenja mogu doprineti prevenciji ili usporavanju progresije ranog stadijuma senilne degeneracije makule ka kasnoj fazi bolesti. Cilj ovog rada je ispitivanje uticaja suplementacije upotrebom sva tri makularna pigmenta na kvalitet vida kod pacijenata sa ranom fazom senilne degeneracije makule. **Materijal i metode.** U prospektivnu studiju je uključeno 15 pacijenata (ukupno 25 očiju) sa ranom senilnom degeneracijom makule. Testovi za procenu kvaliteta vida – vidna oštrina, zablještavanje i osetljivost na kontrast vršeni su na početku praćenja i nakon šest meseci suplementacije sa 10 mg luteina, 2 mg zeaksantina i 10 mg mezozeaksantina. **Rezultati.** Rezultati su pokazali da je osećaj zablještavanja poboljšan kod 20% slučajeva. Kod većine ispitivanih očiju (76%) nije uočeno pogoršanje u vidnoj oštrini. Kontrastna osetljivost je, slično prethodnom, u 80% slučajeva ostala stabilna ili je poboljšana. Nije došlo do statistički značajnih promena nakon šest meseci u odnosu na početne vrednosti kod sva tri ispitivana parametra. **Zaključak.** Nakon šestomesečne suplementacije upotrebom sva tri makularna pigmenta, većina ispitivanih slučajeva (preko 75%) ostala je stabilnog vida ili je uočeno poboljšanje praćenih parametara. Ovaj tip suplemenata poseduje potencijal za očuvanje ili poboljšanje kvaliteta vida kod pacijenata sa ranim stadijumom senilne degeneracije makule.
Ključne reči: makularna degeneracija; oštrina vida; dijetalni suplementi; makularni pigment; beta karoten; lutein; zeaksantin; faktori rizika

Introduction

Age-related macular degeneration (AMD) is one of the most common eye conditions in older population. At the beginning of the 20th century, only a small percentage of population was diagnosed with blindness caused by AMD. Nowadays, due to global population aging, almost half of all cases of blindness in people older than 40 years are related to this disease. The vision loss has dramatic effects on the quality of life and causes loss of social independence, which indicates that AMD should be recognized as a major public health problem [1, 2].

It is believed that inherited and environmental risk factors contribute to AMD. Several studies have shown that genetic factors include locus on chromosome 1 in the complement factor H region as well as locus in the HTRA1/ARMS2 region on chromosome 10 [3–6]. Other inherited features associated with increased risk of AMD include light skin col-

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AMD	– age-related macular degeneration
RPE	– retinal pigment epithelium
MP	– macular pigment
L	– lutein
Z	– zeaxanthin
MZ	– meso-zeaxanthin
MPOD	– macular pigment optical density
VA	– visual acuity
CS	– contrast sensitivity
GD	– glare sensitivity
AREDS	– age-related eye disease study

our, light iris colour and female gender [7 - 9]. On the other hand, prevention of AMD is a growing area of research and it is necessary to identify and alter modifiable risk factors such as smoking, obesity, unhealthy dietary habits, hypertension etc.

Early AMD is characterised by the presence of drusen and/or pigmentary abnormalities (hyper- and/or hypo-pigmentation) at the macula. Typically, early AMD is not associated with any subjective visual complaints. Patients with early AMD should be warned of their increased risk of developing late AMD (dry form - geographic atrophy and wet form – subretinal neovascular membrane). Prevention of late AMD includes cessation of tobacco use, specific diet and supplementation with retinal antioxidants. Also,
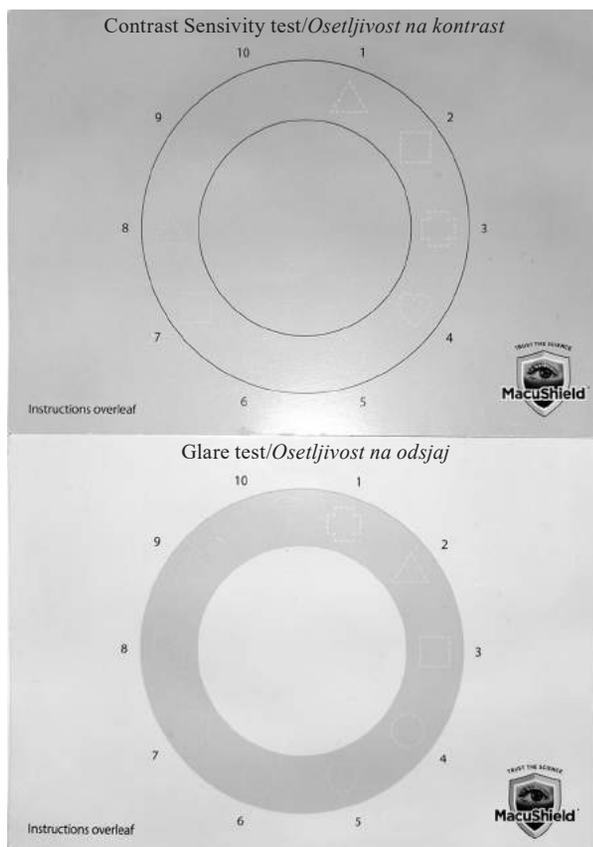
Figure 1. Contrast and glare sensitivity tests
Slika 1. Testovi za kontrastnu osetljivost i odsjaj
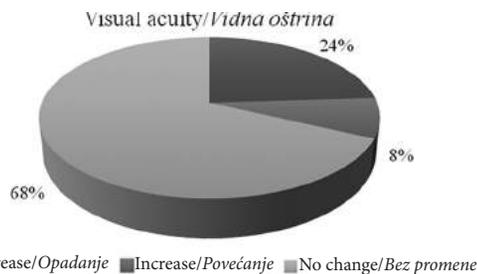
Figure 2. Changes in visual acuity after six months of supplementation
Slika 2. Promene u vidnoj oštrini nakon šest meseci suplementacije

patients should be informed about the symptoms of late AMD (vision loss and metamorphopsia - perception of straight lines appearing wavy) and encouraged to seek medical attention from their ophthalmologist urgently if such symptoms occur, because there is no efficient treatment for impaired vision.

The pathogenesis of this chronic disease includes oxidative stress and inflammation [10, 11] which may lead to loss of central vision, as a consequence of degeneration of photoreceptors and retinal pigment epithelial (RPE) cells. The macula is a specialized part of the retina which accumulates macular pigment (MP), yellow pigment which consists of carotenoids - lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ) [12]. Generally, all three carotenoids are present in equal amounts at the human macula, but their distribution varies depending on the exact location (e.g. MZ is the dominant carotenoid at the fovea). The MP can protect the retina from oxidative stress in two ways - it filters blue light preventing photo oxidative damage in the eye passively. Carotenoids, due to the system of conjugated carbon-carbon double bonds in their structure, act as antioxidants by quenching reactive oxygen species [13, 14]. The extra conjugated double bond makes Z and MZ more stable and better antioxidants compared to L [15, 16]. It is also important to point out that the antioxidant effect of the MPs (L, Z and MZ) is maximized when they are present in the ratio 1 : 1 : 1, than individual carotenoids present at the same total concentration [17]. Evidence also suggests that L and Z can exert anti-inflammatory effects [18, 19].

Carotenoids should be obtained from diet, since they cannot be synthesized by humans [20]. Green leafy vegetables, orange and yellow fruits are rich sources of L [21], while corn and corn products are the most important sources of Z [22]. Human diet is generally poor in MZ, which has been detected only in some sea products (shrimp carapace, fish skin and turtle fat) [23]. Additionally, it is considered that MZ is generated at the macula from L by biochemical transformation [24]. In nature, L is most abundant in the flower petals of yellow Marigold (Tagetes erecta L.) [25], which is often used as a raw material for production of dietary supplements.

There are epidemiological data which indicate that an inverse relation exists between the risk of AMD

Figure 3. Changes in glare sensation after six months of supplementation
Slika 3. *Promene u osećaju odsjaja nakon šest meseci suplementacije*

Figure 4. Changes in contrast sensitivity after six months of supplementation
Slika 4. *Promene u kontrastnoj osetljivosti nakon šest meseci suplementacije*

and the amount of MPs in the retina [26, 27]. Several studies have shown positive association between MP and visual performance parameters such as visual acuity (VA), contrast sensitivity (CS), photo stress recovery and glare sensitivity (GS) [28–30]. Additionally, it has been pointed out that visual parameters could be improved in patients with early form of AMD by supplementation with macular carotenoids [31, 32], although there is still no general recommendation concerning the dosage and composition of supplements.

Therefore, the aim of this study was monitoring the visual parameters (VA, GS and CS) in patients with early AMD during a 6-month period of supplementation with all three macular carotenoids (L, Z, and MZ).

Material and Methods

This prospective study initially included 30 patients, while the complete 6-month follow up was performed in 15 patients, during 2018. A total of 25 eyes of 15 patients with non-exudative early AMD (small hard drusen, soft drusen and hypo/hyper pigmentation disorders) and no evidence of choroidal neovascularization were analyzed.

All the tests were performed at the beginning of the study and after 6 months of continuous supplementation with 10 mg L, 2 mg Z, and 10 mg MZ soft gel capsules (commercially available dietary supplement). Patients were instructed to take one capsule daily with a glass of water after breakfast. Detailed explanations of the study procedures were provided to all the participants.

The VA examination at distance - the best corrected (Snellen chart), VA at near, CS test, and GS test at near were measured (**Figure 1**) (Macushield test). External slit lamp examination, intraocular pressure and noncontact fundus examination with Volk lens 90/68 D after mydriasis were performed. Optical coherent tomography (OCT) and macular thickness were analyzed to verify the type of degenerative changes.

Table 1. Baseline characteristics of patients included in the study
Tabela 1. *Karakteristike pacijenata uključenih u studiju na početku ispitivanja*

Characteristics/*Karakteristike*	N/*Broj* = 15
Age (y)/*Starost (g)*	
51 – 60	1
61 – 70	6
71 – 80	8
Sex/*Pol*	
Male/*Muški*	7
Female/*Ženski*	8
Body mass (kg)/*Telesna masa (kg)*	60–120
Smoking habits/*Pušenje*	
Current/*Trenutno*	6
Past/*Ranije*	1
Never/*Nikad*	8
Diabetes/*Dijabetes*	1
Arterial hypertension/*Arterijska hipertenzija*	3
Eye disorders/*Bolesti oka*	
Cataract/*Katarakta*	4
Glaucoma/*Glaukom*	3
Myopia/*Miopija*	2
Lipid disorders/*Poremećaji lipida*	7

Table 2. Values of examined visual parameters at baseline (0) and after six months (6) of supplementation
Tabela 2. Vrednosti ispitivanih parametara na početku studije (0) i nakon šest meseci (6) suplementacije

	VA 0	VA 6	GS 0	GS 6	CS 0	CS 6
mean/value/*srednja vrednost*	0.80	0.77	6.04	6.00	5.96	5.88
SD	0.20	0.23	2.61	2.80	2.56	2.95

Legend: VA – visual acuity; CS – contrast sensitivity; GS – glare sensitivity; SD – standard deviation
Legenda: VA – vidna oštrina; GS – osetljivost na odsjaj; CS – kontrastna osetljivost; SD – standardna devijacija

The patients' pupils were previously dilated with 1% tropicamide and 10% phenylephrine.

Statistical analysis of the data comparing the changes from baseline and 6 months after administration of the supplement was performed using Student t-test ($p < 0.05$) in Microsoft Excel program.

Results

A total of 25 eyes of 15 patients with early AMD at baseline and after 6 months of supplementation were analyzed. The mean age of study subjects (7 male and 8 female) was 71 ± 6.9 (± 1 SD) years. Seven patients were active smokers, one had diabetes type 2, and three patients had arterial hypertension. Lipid disorders were diagnosed in seven patients (hypercholesterolemia and/or elevated triglycerides). The measured body weight was from 60 to 120 kg (80.27 ± 17.28 kg, on average). Non-significant lens opacifications were detected in four patients. Three patients had compensated open angle glaucoma and two patients had mild myopia (**Table 1**).

The best corrected VA at baseline was 0.80 and after six months it was 0.77 on average, with no statistically significant difference ($p < 0.05$) (Snellen chart) (**Table 2**). In most of the examined eyes (68%) no changes in VA were observed, improvement was detected in 8%, while mild worsening was found in 24% of cases (**Figure 2**). It may be concluded that 76% of the examined eyes stayed stable during six months of examination.

There were no statistically significant changes ($p < 0.05$) in measured GS after 6 months of supplementation compared to baseline (**Table 2**). Individually, in 20% of cases better results were noticed, while in the majority of examined eyes (64%) no changes were detected (**Figure 3**).

Similar results were obtained using the CS test – in 80% of patients vision quality remained stable (increase or no change in CS) (**Figure 4**).

Discussion

In the present study, patients' age ranged from 56 to 80 (71 years, on average). The majority of the examined patients (53.3%) were in the group from 71 to 80 years, which confirms that aging is one of the leading risk factors for AMD. Besides aging, there are other non-modifiable factors which affect AMD like light skin, female gender, hyperopia or a family history of hyperopia [33] and patients with these pre-

dispositions should be advised to visit their ophthalmologist regularly. Body weight of the subjects varied greatly - from 60 to 120 kg. Almost half of the patients had some kind of lipid disorder and six from fifteen patients (40%) were active smokers. These factors are considered to be the leading causes of AMD. Additionally, obesity, poor dietary habits, hypertension and diabetes are recognized as other modifiable risk factors [34]. Bearing in mind that only one of followed patients in our study had diabetes type 2 and three had hypertension, we should point out that in this group of patients - age, lipid disorders and smoking were the most frequent risk factors.

Our results showed that more than 75% of followed patients remained stable or had certain improvement of vision characteristics. The best results were obtained for GS test where 20% of cases showed improvement, and 64% of cases had no change in vision quality. Although this study shows no statistically significant improvement of visual performance, it should be emphasized that for all analyzed parameters decrease of vision quality was detected in less than 25% of examined subjects. Additionally, adverse effect of supplementation was not noticed, and none of the tested patients developed late phase of AMD during the follow-up. Study of Sabour-Picket et al. confirmed enhancement of CS and increase of macular pigment optical density (MPOD) after a 12-month supplementation with all three MPs, compared to supplementation only with L and Z [35]. Another published paper confirmed improvements in VA, CS and MPOD after 6-month supplementation with L, Z and MZ (10/2/10 mg, respectively). Mentioned effects were not observed in subjects receiving supplement with 20 mg of L and 2 mg of Z [36]. These studies also corroborate that the supplementation containing all three macular carotenoids is the most efficient in a combination of 10 mg of L, 2 mg of Z, and 10 mg of MZ. Moreover, it should be emphasized that formulation content and composition is crucial for obtaining optimal bioavailability of macular carotenoids. Namely, the study performed by Meagher et al. (2013) reported results concerning serum response to three different carotenoid supplements (Group 1 – 20 mg L and 2 mg Z; Group 2 – 10 mg L, 2 mg Z and 10 mg MZ; Group 3 – 3 mg L, 2 mg Z and 17 mg MZ). It was concluded that the greatest serum response of total carotenoids was obtained in Group 2, leading to the highest potential delivery to target tissue. Although the total intake of carotenoids in Group 2 and 3 was the

same, serum levels of L and Z in Group 3 did not significantly change compared to baseline. This could be due the fact that high levels of MZ can potentially inhibit the absorption of other carotenoids. Additional analysis of supplements applied in Group 1 showed that it contained MZ although this compound was not declared on the label [37].

The prevention and reduction of risk factors leading to advanced AMD is the general approach in the treatment of this disease. Hence, different studies were performed to evaluate the effects of supplementation with antioxidants, vitamins and minerals in patients with AMD. The Age-Related Eye Disease Study (AREDS) was the first large clinical trial conducted between 1992 and 2001. The study followed 3.640 individuals for over 6 years (on average). Supplementation included: 500 mg of vitamin C; 400 IU of vitamin E; 15 mg of beta-carotene; 80 mg of zinc oxide, and 2 mg of cupric oxide. The general conclusion was that intake of this combination of vitamins and minerals leads to risk reduction of advanced AMD by about 25% [38]. The following AREDS 2 study, performed between 2006 and 2008, included even more participants across the United States. The main difference in supplementation compared to AREDS 1 was elimination of beta-carotene (inducing lung carcinoma in active smokers) and reduction of zinc level (from 80 to 25 mg). Also, AREDS 2 formulation contained 10 mg of L, 2 mg of Z, and 1.000 mg of omega 3 fatty acids [39].

Recent studies pointed out the importance of macular carotenoid – MZ in vision enhancement and MP increase in patients with non-advanced AMD [35, 36, 40, 41]. The meta-analysis published by Ma et al. (2016) showed positive effects of L, Z and MZ supplementation on MPOD augmentation both in AMD patients and healthy volunteers [41]. Also, it was noticed that addition of MZ resulted in greater increase in MP. Observed improvement was correlated with the increase of xanthophyll carotenoids concentration in blood. Connolly et al. (2011), analyzed serum and macular response to supplementation with all three macular carotenoids. A statistically significant increase in serum concentration of these carotenoids and increase in central MPOD was established. Also, no adverse effects or toxicity were noticed after consumption of these compounds [40].

Of course, the presented study should be continued, because the enhancement of vision quality requires more time, especially taking into account the condition of patient's vision at baseline. Also, in this type of clinical study, compliance is very important and it could be improved by more frequent contact with patients. Also, a limitation of our study is absence of a placebo group. Application of more sensitive tests could give more accurate results. For example, modern techniques such as Macular Pigment Densitometer for measuring MPOD also give better insight into the pathology and condition of macula, which is one of the most reliable parameters for quantitative assessment of macular status. Additionally, it is recommended that qualitative and quantitative analysis of applied supplement is performed, in order to confirm if it corresponds with the manufacturer's label declaration.

Conclusion

This study showed that subjects supplemented with all three macular carotenoids (10 mg lutein, 2 mg zeaxanthin, and 10 mg meso-zeaxanthin) presented with improvement of followed parameters in 8–20% of cases, and the majority (64–68%) remained stable during the 6-month period. In the examined patients, besides age, lipid disorders and smoking were the most frequent risk factors for age-related macular degeneration. No adverse effect of supplementation was noticed, and none of the tested patients developed late phase age-related macular degeneration. The present study should be continued with a larger number of participants, better compliance and inclusion of a placebo group. Also, there are still concerns about the bioavailability of macular carotenoids and their long term safety.

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CORRELATION BETWEEN ATMOSPHERIC PRESSURE CHANGES AND INCIDENCE OF ABDOMINAL AORTIC ANEURYSM RUPTURES

KORELACIJA IZMEĐU ATMOSFERSKOG PRITISKA I INCIDENCIJE RUPTURE ANEURIZME ABDOMINALNE AORTE

Miloš KAĆANSKI[1], Slavko BUDINSKI[1, 2], Vladimir MARKOVIĆ[1 ,2] and Božidar DEJANOVIĆ[2,3]

Summary

Introduction. Abdominal aortic aneurysm and its rupture have been often studied over the years, and numerous risk factors for aneurysm rupture have been considered, including atmospheric pressure. The effect of atmospheric pressure has not yet been sufficiently investigated, although studies have been conducted on its possible impact on aneurysms. The aim of this study was to determine the presence of comorbidity as well as the impact of atmospheric pressure on the incidence of abdominal aortic aneurysm ruptures. **Material and Methods.** All patients who underwent abdominal aortic aneurysm rupture at the Clinic of Vascular and Endovascular Surgery of the Clinical Center of Vojvodina in the period from January 1, 2014 to December 1, 2018 were included in this study. The retrospective study was conducted using patient medical records, computer data, additional clinical records, and discharge reports. Data on atmospheric pressure in the territory of Vojvodina were collected from the Republic Hydrometeorological Institute of Serbia. **Results.** The number of cases with abdominal aortic aneurysm rupture was found to be higher at higher atmospheric pressure than expected at that altitude, in as much as 95% of cases, most often in the fall. **Conclusion.** We concluded that there is a significant correlation between high atmospheric pressure and the incidence of abdominal aortic aneurysm rupture, but the exact mechanism of this impact remains to be studied in some further studies.
Key words: Aorta, Abdominal; Aortic Aneurysm; Aneurysm, Ruptured; Aortic Aneurysm, Abdominal; Risk Factors; Atmospheric Pressure; Seasons; Comorbidity; Mortality; Morbidity

Sažetak

Uvod. Često se tokom godina proučava aneurizma abdominalne aorte i njena ruptura, a razmatrani su i brojni faktori rizika za rupturu aneurizme, među kojima i atmosferski pritisak. Uticaj atmosferskog pritiska još uvek nije dovoljno istražen, iako su rađene studije o njegovom mogućem uticaju na aneurizme. U ovom radu smo hteli da utvrdimo prisustvo komorbiditeta, kao i uticaj atmosferskog pritiska na učestalost rupture aneurizme abdominalne aorte. **Materijal i metode.** Svi pacijenti kojima je operisana ruptura aneurizme abdominalne aorte na Klinici za vaskularnu i endovaskularnu hirurgiju Kliničkog centra Vojvodine u period od 1.01.2014. do 1.12.2018. godine uključeni su u ovu studiju. Analiza je retrospektivna. Koristili smo istoriju bolesti bolesnika, medicinsku dokumentaciju (kompjuterske podatke, prateću kliničku dokumentaciju, otpusne liste) za dobijanje podataka. Podaci o atmosferskom pritisku na teritoriji Vojvodine prikupljeni su od Republičkog hidrometeorološkog instituta Srbije. **Rezultati.** Utvrđeno je da je broj slučajeva sa rupturom aneurizme abdominalne aorte veći pri većem atmosferskom pritisku od očekivanog na toj nadmorskoj visini, čak u 95% slučajeva, najčešće na jesen. **Zaključak.** Zaključili smo da postoji korelacija između visokog atmosferskog pritiska i incidencije rupture aneurizme abdominalne aorte, a tačan mehanizam tog uticaja ostaje da se prouči u nekim daljim studijama.
Ključne reči: abdominalna aorta; aneurizma aorte; ruptura aneurizme; aneurizma abdominalne aorte; faktori rizika; atmosferski pritisak; godišnja doba; komorbiditet; mortalitet; morbiditet

Introduction

The abdominal aorta is the main blood vessel in the abdominal cavity; it is located in the retroperitoneal space and its normal diameter is less than 2 cm. The abdominal aortic aneurysm (AAA) is defined as a dilatation of the aorta to the diameter that is at least 50% greater than the normal. Approximately 80% of aortic aneurysms are located below the renal arteries, commonly involving the infrarenal segment [1]. A large number of AAAs are asymptomatic, and cannot be detect by physical examination, so they remain undiscovered until they are accidentally detected on ultrasonography (US). Symptomatic AAA presents as a pulsating abdominal mass, followed by back pain, and pain in the abdomen, genitals and along the leg. The most severe complication of AAA is a rupture (rAAA), accounting for over 50% of deaths, and the regulation of risk factors is very important [2].

Computed tomography (CT) has been and remained a gold standard for the diagnosis of rAAA. However, in recent years, US has been widely used,

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Abbreviations

AAA   – abdominal aortic aneurysm
rAAA   – ruptured abdominal aortic aneurysm
US   – ultrasonography
CT   – computed tomography
HTA   – hypertension
DM   – diabetes mellitus
CMP   – cardiomyopathy
COPD – chronic obstructive pulmonary disease
AP   – atmospheric pressure
CVI   – cerebrovascular insult
RHIS   – Republic Hydrometeorological Institute of Serbia

and is often used as a method of choice in urgent conditions where rapid and accurate diagnosis of suspected rAAA is required in patients in critical general condition [3]. Surgical repair of AAA is indicated when the transverse diameter is > 55 mm in males and > 50 mm in females, or if the dilatation is 1 cm for 1 year or 0.5 cm for 6 months, since then the patient is at increased risk for rAAA [4]. Smoking (nicotinism), hypertension (HTA), diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), cardiomyopathy (CMP) are just some (but highly significant) risk factors for the development and prognosis of rAAA. One of the risk factors for rAAA is atmospheric pressure (AP), but there are still no studies to directly correlate and prove the change in AP and the incidence of rAAA, as well as the exact flow of those mechanisms. Changes in weather conditions are associated with cardiovascular diseases, such as acute myocardial infarction (AIM), cerebrovascular insult (CVI), and rAAA. Atmospheric pressure represents the weight of the air column above the unit surface. The basic unit is Pascal, and the average sea-level pressure is 1013.25 mbar [5].

Since the patients included in this study stayed on the territory of Vojvodina at the moment of rAAA, their place of residence was at an altitude of 80 m, the AP value of 1005.25 mbar was used as a standard.

The association between AP and rAAA can be indirectly explained by the Laplace's law, which implies that the aneurysm wall tension can be defined by the aneurysm diameter and pressure differences in the aneurysm sac and pressure outside it. With constant transmural pressure, the wall tension is directly proportional to the radius of the aneurysm. In this way, Laplace's law highlights the importance of the size of the aneurysm, as well as the HTA on the rupture of
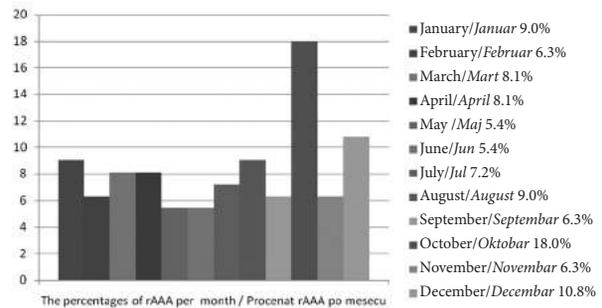
Graph 1. The percentages of patients with abdominal aortic rupture per month
Grafikon 1. Procenat pacijenata sa rupturom aneurizme abdominalne aorte po mesecu

the aneurysm. Because of this, AP changes are often associated with precipitation and rupture of AAA [6].

The aim of the study was to analyze the presence of comorbidity in patients with rAAA, to analyze the influence of AP on the incidence of rAAA, and establish the connection between AP and the length of survival of patient after aortic surgery.

Material and Methods

The retrospective study included 111 patients who underwent surgery for rAAA in the period from from January 1, 2014 to December 1, 2018. The patients included in this study were admitted and operated at the Clinic of Vascular and Endovascular Surgery under the diagnosis of rAAA. The rAAA is defined as an aneurysmal wall defect associated with blood extravasation and consequential retroperitoneal hematoma formation or with the presence of blood in the peritoneum, confirmed by a CT scan, with or without US. The data were obtained from patient medical histories, medical records (computer database, accompanying clinical documentation, discharge records). Due to the inability to reliably define the exact time of rupture, patients with aortocaval (or aortorenal) fistula, as well as patients with signs of chronic contained rAAA, were excluded from the study. Patients with thoracoabdominal aneurysm were also excluded from the study.

Correlations between rAAA monthly incidence and monthly mean AP were assessed using Pearson's correlation coefficient. Statistically significant difference was set at $p < 0.05$. Statistical analysis

Table 1. Number of patients with abdominal aortic aneurysm rupture in regard to the atmospheric pressure in Serbia, in the territory of Vojvodina
Tabela 1. - Broj pacijenata sa rupturom aneurizme abdominalne aorte u odnosu na visinu atmosferskog pritiska u Srbiji, na teritoriji Vojvodine

Atmospheric pressure in the territory of Vojvodina *Atmosferski pritisak za teritoriju Vojvodine*	Number of patients with rAAA *Broj pacijenata sa rAAA*
High/*Visok* (above/*iznad* 1008.25 mbar)	105
Normal/*Normalan* (1005.25 mbar ± 3)	5
Low/*Nizak* (under/*ispod* 1002.25 mbar)	1

was performed using SPSS for Windows v. 21.0 (SPSS Inc. Chicago, IL). The study, as well as the consent to use patients' medical records, was obtained from the Ethics Committee of the Faculty of Medicine in Novi Sad. During the conduct of this research, all ethical principles were respected.

Atmospheric pressure parameters

All patients were from the territory of Vojvodina, and all data on atmospheric pressure were collected from the Republic Hydrometeorological Institute of Serbia (RHIS). The AP values for the place of residence and the date of receipt for all patients were calculated to get an arithmetic mean of 4 time intervals. These data consisted of AP values in the period from January 1, 2014 to December 1, 2018, measured at 4 time intervals: at 6 am, 12 am, 6 pm and 12 pm with coordinates 45° 15'6 '' N, 19° 50'13 '' E and altitude of 80 m, with normal atmospheric pressure of 1005.25 mbar, taken as a standard in this study.

Results

Annual and seasonal variability

From January 2014, to December 2018, 111 patients were treated for rAAA, of which 86 were males (77.54%) and 25 were females (22.53%). The average age of male patients was 68 years, and of female patients 76 years; on average, every 16 days a patient underwent surgery for rAAA.

On the annual level, including all 12 months, in 95.5% of cases, the rAAA occurred when the atmospheric pressure was above the limit of 1005.25 mbar. This atmospheric pressure was taken as a standard in this paper because of the altitude of 80 m which is the average value for Vojvodina, the territory of Serbia.

The statistical data processing of AP values and dates of rAAA occurrence showed that the month with the highest rAAA percentage was October, accounting for 18.01% of all rAAA cases, whereas May and June accounted for the lowest rate of rAAA with 5.40%.
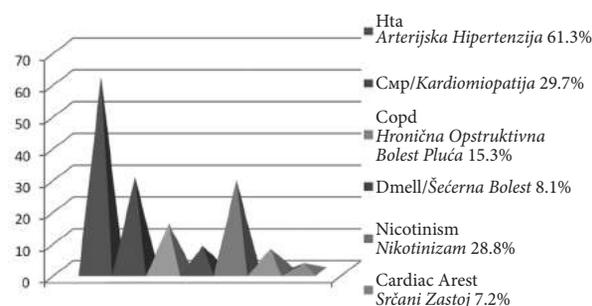
Graph 2. The percentage of comorbidities, cardiac arrests, and lethal outcomes in patients with abdominal aortic aneurysm rupture
Grafikon 2. Procenat komorbiditeta, zastoja srca i smrtnog ishoda kod pacijenata sa rupturom aneurizme abdominalne aorte

We also found that the incidence of rAAA was increasing every year, which may be due to a prolonged lifespan in general.

Morbidity and mortality

Further, the relationship between rAAA and associated diseases was analyzed. We have noticed that HTA, as one of the most significant risk factors, was found in 61.3%. The CMP was found in 29.7%, COPD in 15.3%, nicotinism in 28.8% and DM in 8.1% of the examined cases of rAAA. We also studied the course of the surgery, cases of cardiac arrest, and lethal outcome, which is a possible outcome due to hypovolemic shock and consequent hemodynamic instability in rAAA. The cardiac arrest rate was 7.2%, while fatal intraoperative outcome occurred in 2.7% of all rAAA cases.

Also, retroperitoneal bleeding was found in 82.9% and intraperitoneal in 17.1% of cases. In regard to the survival data, the following results were obtained: 27.02% of patients survived for 7 days after surgery, 6.30% of patients survived up to 14 days, 9.00% up to 30 days, and 57.65% of all rAAA cases survived for more than 30 days and were released for home treatment; this leads to the conclusion that if a patient survived the first 7 days after the occurrence and treatment of rAAA, in 78% of cases he was released for home treatment. Further analysis of AP and survival rate, based on the Pearson's correlation coefficient, showed that there was no statistically significant relationship between high AP and the survival of patients (p > 0.05).

Discussion

So far, a small number of papers have studied the impact of AP on potential rAAA. The largest study, to this day, was conducted in France including 494 inpatients; it established 6,358 deaths due to rAAA and also showed that high AP was preceded by rAAA by 30 days [7]. The problem researching this subject is that the majority of scientific papers and studies cannot be adequately connected, because AP is tested in different geographical areas, and it is impossible to get a definitive conclusion on whether the change of AP really plays a role in the mechanism and incidence of rAAA. Also, considering only AP changes, other weather parameters that may impact rAAA are excluded. That is why it remains to be studied in detail and proven which AP mechanism affects rAAA [8].

In this study, we explored if there is a correlation between different values of AP and rAAA. In addition, we also investigated other comorbidities, risk factors for rAAA, as well as seasonality. This paper included a group of patients, of whom 77.54% were males, 22.53% females that corresponded to previous studies [9]. In regard to age distribution, the average age of males was 68 years, and 76 years of females. The statistical data analysis of AP values and rAAA dates showed that the month with the highest percent-

age of rAAA was October with 18.01% of 111 cases of rAAA, and that May and June were the months with the lowest percentage of rAAA with 5.40% of rAAA cases. On the annual level, including all 12 months, in 95.5% of cases of rAAA, the AP was high above the normal limit of 1005.25 mbar.

Also, one of the earlier studies reported that AP was high 3 – 7 days before, as well as 3 days after rAAA for a given territory [10]. The results of such studies have contributed to supporting the results and conclusions in this paper, where we found that the month with the highest rAAA was October with 18.01%, and where the average AP, according to the data of the RHIS, for a 5-year period that we investigated, was 1018,20 mbar, which was higher than the arithmetic average 1005,25 mbar taken as a standard for AP for the territory of Vojvodina.

Takagi and associates conducted an analysis of more than 14 studies including over 3,978 patients with rAAA and came to a similar conclusion like we did in this study: the seasons with the highest number of rAAA are autumn and winter, accounting for 18.01% of rAAA in October, 10.81% in December, and 9.00% of rAAA in January [11]. The reason why the incidence of rAAA is highest in autumn and winter is the fact that people spend more time indoors, when they are exposed to nicotinism, which again significantly affects the potential occurrence of rAAA [12]. Also, our region is well known for dietary changes during winter, with high fat and salt intake, as well as drinking alcohol and reduced physical activity, that may result in increased arterial pressure and deterioration of already present hypertension [10].

The analysis of comorbidities showed that HTA, as the most significant risk factor, was present in 61.3% of patients with rAAA, and it was followed by CMP in 29.7% of rAAA patients, COPD in 15.3%, nicotinism in 28.8% and DM in 8.1% of rAAA cases. Such results correspond with the literature data, reporting that the most important risk factors of rAAA were nicotinism and hypertension [13]. Moreover, these results are supported by histological and biochemical changes occurring in rAAA. Histologically speaking, changes that are found in AAA include low arterial elasticity as well as aortic dilatation, increased levels of collagen, decreased smooth muscle cell density, and a large number of inflammatory cells. Except for these changes, we can also find elevated values of inflammatory markers, such

as interleukin-6 (proinflammatory cytokine) and interleukin-10 (anti-inflammatory cytokine) [14].

Elevated inflammatory markers may, among other things, be associated with comorbidities, especially nicotinism and COPD, which can, in combination with HTA, due to the already explained law of Laplace, lead to a higher number of rAAA in patients with AAA and associated diseases. The COPD is often associated with cardiovascular diseases, including AAA, which supports the finding of COPD in 15.3% of the examined patients in this study. The reasons for the connection have not yet been thoroughly examined, but it can be due to systemic inflammation, chronic infections, common risk factors, such as nicotinism, that can be found in both COPD and rAAA [15].

In this study, diabetes was found in 8.1% of patients and it corresponds to findings of a study investigating the relationship between DM and rAAA, which reported that in 188 patients with rAAA there were only 6.4% of diabetic patients [16]. Of the 111 cases of rAAA in this study, the occurrence of perioperative cardiac arrest and exitus letalis, as a consequence hypovolemic shock and consequent hemodynamic instability in rAAA were reported in 7.2% and 2.7% of patients, respectively. The percentage of bleeding sites in rAAA was also calculated and retroperitoneal hematoma was found in 82.9% of cases, whereas intraperitoneal was reported in 17.1%, which corresponds to data about rAAA, where the most common site of bleeding, as well as hematoma, is the retroperitoneal space. The data on bleeding were supported by Karthikesalingam and associates, who examined the spectrum of CT findings in rAAA, and concluded that the most characteristic finding, as well as the most common bleeding site, was the retroperitoneal space [4].

Conclusion

The conclusion of this study is that less comorbidity is associated with lower periprocedural mortality and morbidity, and that high arterial blood pressure is the leading comorbidity in patients with ruptured abdominal aortic aneurysm. We also concluded that there is a significant connection between high atmospheric pressure and abdominal aortic aneurysm rupture, and that there is no statistically significant correlation between high atmospheric pressure and the length of survival.

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PARENTAL ATTITUDES TOWARDS THE USE OF HUMANOID ROBOTS IN PEDIATRIC (RE)HABILITATION

STAVOVI RODITELJA O PRIMENI HUMANOIDNIH ROBOTA U DEČJOJ (RE)HABILITACIJI

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Summary

Introduction. The advancement of technology in the world is more and more evident, as is its implementation in almost all spheres of life, and so is its application in medicine. The use of robotics in medicine has become increasingly important, with a wide scope of application. Thus, the aim of this study was to determine parental attitudes towards the robot-assisted therapy in pediatric (re)habilitation and to assess the degree of parental acceptance and apprehension towards humanoid robots, along with their expectations. **Material and Methods**. The study included 32 parents of both genders, whose children took part in the treatment using a mobile anthropomorphic robot with cognitive skills, and who completed the Frankenstein Syndrome Questionnaire. **Results.** The study showed that parental attitudes towards humanoid robots and robot-assisted therapy were positive. **Conclusion.** In our study, parents showed neutral attitudes and positive expectations of humanoid robots. They have accepted them socially, without negative feelings on the religious and psychosocial levels.
Key words: Communication; Robotics; Therapy, Computer-Assisted; Social Perception; Health Knowledge, Attitudes, Practice; Parents; Child; Rehabilitation; Surveys and Questionnaires

Sažetak

Uvod. Napredak tehnologije u svetu je svakodnevno sve očigledniji kao i njena implementacija u skoro svim sferama života, pa je tako i s njenom primenom u medicini. Robotika u medicini je sve značajnija, a područje primene je veoma široko. Ciljevi ovog rada bili su da se utvrdi stav roditelja o primeni robot-asistirane terapije u dečjoj (re)habilitaciji i da se proceni stav roditelja prema humanoidnim robotima, njihova očekivanja i stepen straha. **Materijal i metode**. Istraživanje je obuhvatilo 32 roditelja oba pola, čija su deca bila uključena u tretman sa humanoidnim robotom Markom, i koji su popunjavali *Upitnik sa Frankeštajn sindromom*. **Rezultati.** Istraživanje ukazuje da je stav roditelja prema primeni humanoidnih robota kao i robot-asistirane terapije pozitivan. **Zaključak.** U našoj sredini roditelji imaju neutralan stav i pozitivna očekivanja od humanoidnih robota; prihvatili su ih − nemaju negativna osećanja na religioznom i psihosocijalnom nivou.
Ključne reči: komunikacija; robotika; kompjuterski asistirana terapija; socijalna percepcija; znanje o zdravlju, stavovi, praksa; roditelji; dete; rehabilitacija; istraživanja i upitnici

Introduction

Rapid advances in the field of technology have resulted in their implementation in almost all life domains, including medicine. The use of robotics in medicine has become increasingly important, and its scope of implementation is very broad. Therapeutic use of robots is a relatively recent phenomenon, as it necessitates a certain degree of technological advancement, as well as a high degree of safety and reliability to provide suitability for use with patients, especially children [1–3]. The multidisciplinary approach is one of the core principles of modern medicine related to the field of medical robotics [4].

The term robot was first used by Karel Capek in his play "Rossum's Universal Robots" (R.U.R) in 1921 [5]. It derives from a Czech word "robota" meaning "forced labor" or "hard labor." On the other hand, ro-

botics was first mentioned in the short story "Runaround" authored by the American writer Isaac Asimov in 1942 [5].

Humanoid robot is the term used for a machine that looks and behaves like a human, based on the principles of artificial intelligence (AI) [6]. The advantages of using robots in medicine include higher work quality and productivity, increased safety and risk reduction, and greater operational flexibility [7]. In sum, robots can enhance quality of life and output, while reducing medical costs [8]. Moreover, robots have been shown to improve patient motivation and thus outcome of rehabilitation [9]. Robotics in medicine is presently classified into surgical, medical, service and rehabilitation categories [4].

The first humanoid robot that has been introduced into medical practice in our country is MARKO (acronym for Mobile Anthropomorphic

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Robot with Cognitive Characteristics) and it has since been used in the treatment of children affected by cerebral palsy [10].

The term "Frankenstein syndrome" was first described by Isaac Asimov, who defined it as fear of robots [11]. However, its origins can be traced back to the early 1900s, when Mary Shelley published her novel Frankenstein, or the Modern Prometheus (1818). At the time, people were apprehensive of technology and artificial beings, which they perceived as a threat to humanity [11–13].

Frankenstein syndrome is a term used for assessing acceptance of humanoid robots, including extent of anxiety, i.e. Frankenstein Syndrome Questionnaire (FSQ) [14].

The aim of the present study was to determine parental attitudes towards robot-assisted therapy as part of pediatric (re)habilitation, i.e., corrective exercise program, and to assess parental views of humanoid robots, their expectations and degree of apprehension.

Material and Methods

This prospective study was part of a research project (No III44008) approved by the Ministry of Education, Science and Technological Development of the Republic of Serbia and by the Ethics Committee of the Institute of Child and Youth Health Care of Vojvodina.

The study included 32 parents (N = 32), male and female, whose children had been referred to a two-week-long corrective exercise training program due to poor posture, and who agreed to participate in a course facilitated by humanoid robot MARKO. The research was conducted at the Clinic of Child Habilitation and Rehabilitation at the Institute of Child and Youth Health Care of Vojvodina in Novi Sad, between December 17, 2018 and December 28, 2018.

All participating parents completed the FSQ. The FSQ is an instrument that has been translated and linguistically adapted for the purpose of this investigation, and is used to obtain information pertaining to respondents' attitudes and degree of acceptance of humanoid robots. It includes two sections, the first of which is related to general information such as age, gender and profession, while the second contains 30 items on humanoid robots rated on a 7 point Likert scale (1 - Strongly disagree, 2 - Disagree, 3 - Slightly disagree, 4 - Not sure, 5 - Slightly agree, 6 - Agree, 7 - Strongly agree).

Statistical data analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 21. For quantitative variables, average values (arithmetic mean) and variability (range and standard deviation - SD) were reported, while for qualitative variables, frequencies and percentages were calculated. Quantitative between-group comparisons were performed via nonparametric Mann-Whitney test, whereas correlations were determined through Pearson correlation coefficient, with $p < 0.05$ set as statistically significant.

Results

The study sample included 32 parents of both genders, 25 (78.1%) women and 7 (21.9%) men. The youngest parent was 27, and the oldest 67 years old, with the average age of 41 years.

The greatest number of participating parents (n = 16.50%) had a university degree (UD), while 14 (43.8%) had a high school degree (HSD), and the remaining 2 (6.3%) had only completed a primary school (PS).

When completing the FSQ, parents first responded to questions on their attitudes towards humanoid robots. The majority (n =19.59%) of respondents indicated that they have seen humanoid robots in the media, while 7 (21.9%) reported seeing them in person, and the remaining 6 (18.8%) had never seen one.

All the FSQ items required responses on a 7 point Likert scale. However, for ease of interpretation, these were grouped into three subscales, pertaining to the degree of negative feelings towards humanoid robots (Subscale 1), positive expectations (Subscale 2), and social acceptance of humanoid robots (Subscale 3).

The minimum and maximum ratings on Subscale 1 were 2 and 6, respectively, with the average of 4.06, indicating a neutral attitude towards humanoid robots **(Table 1)**. For the Subscale 2, 2 was the minimum and 7 the maximum rating, resulting in the average score of 5.31, indicating that parents mostly had positive expectations of humanoid robots **(Table 1)**. Finally, the minimum 1 and maximum 5 rating on Subscale 3, with the average of 1.88, suggested that humanoid robots were socially accepted by the parents, in the sense that they had no negative feelings on the religious or psychosocial levels **(Table 1)**.

Table 2 shows the educational attainment for each subscale, whereby statistically significant differences are only noted for the subscale measuring negative feelings towards humanoid robots (Subscale 1). It can also

Table 1. Distribution of the sample regarding the results obtained using the Frankenstein Syndrome Questionnaire (FSQ)
Tabela 1. Raspodela uzorka u odnosu na rezultate prema Frankenštajn sindrom upitniku (FSQ)

Attitudes/*Stavovi*	N/*Broj*	Min/*Min*	Max/*Maks*	Average/*Prosek*	SD
Subscale 1/*Supskala 1*	32	2	6	4.06	0.840
Subscale 2/*Supskala 2*	32	2	7	5.31	1.148
Subscale 3/*Supskala 3*	32	1	5	1.88	1.185

Table 2. Sample distribution by education levels in regard to subscales
Tabela 2. Raspodela uzorka prema stručnoj spremi u odnosu na supskale

Attitudes *Stavovi*	EL*	N *Broj*	Average *Prosek*	SD	Min *Min*	Max *Maks*	Mann Whitney test U *Man Vitnijev U test*	P
Subscale 1 *Supskala 1*	PS† and HSD‡/*OSS i SSS*	16	4.38	0.806	3	6		
	UD**/*VSS*	16	3.75	0.775	2	5	81.000	0.045
	Total/*Ukupno*	32	4.06	0.840	2	6		
Subscale 2 *Supskala 2*	PS† and HSD‡/*OSS i SSS*	16	5.38	1.025	3	7		
	UD**/*VSS*	16	5.25	1.291	2	7	125.500	0.922
	Total/*Ukupno*	32	5.31	1.148	2	7		
Subscale 3 *Supskala 3*	PS† and HSD‡/*OSS i SSS*	16	2.00	1.366	1	5		
	UD**/*VSS*	16	1.75	1.000	1	4	120.500	0.754
	Total/*Ukupno*	32	1.88	1.185	1	5		

Legend/*Legenda*: *Education Level/*Stepen stručne spreme*; †Primary school/*Osnovna stručna sprema*; ‡High School Degree/ *Srednja stručna sprema*; **University degree/*Visoka stručna sprema*

Table 3. Gender distribution of the sample in regard to subscales
Tabela 3. Raspodela uzorka prema polnoj strukturi u odnosu na supskale

Attitudes *Stavovi*	Gender *Pol*	N *Broj*	Averege *Prosek*	SD	Min *Min*	Max *Maks*	Mann Whitneytest U *Man Vitnijev U test*	P
Subscale 1 *Supskala 1*	Male/*Muško*	7	3.71	0.756	2	4		
	Female/*Ženski*	25	4.16	0.850	3	6	69.000	0.341
	Total/*Ukupno*	32	4.06	0.840	2	6		
Subscale 2 *Supskala 2*	Male/*Muško*	7	5.43	0.976	4	7		
	Female/*Ženski*	25	5.28	1.208	2	7	86.000	0.943
	Total/*Ukupno*	32	5.31	1.148	2	7		
Subscale 3 *Supskala 3*	Male/*Muško*	7	1.57	1.134	1	4		
	Female/*Ženski*	25	1.96	1.207	1	5	70.500	0.390
	Total/*Ukupno*	32	1.88	1.185	1	5		

be observed that less educated parents showed more negative attitudes towards humanoid robots compared to those holding a university degree **(Table 2).**

In regard to gender distribution, no statistically significant differences were seen in any of the three subscales, as shown in **Table 3**. Finally, when responses in the three subscales were examined in relation to the examinees' age, no statistically significant differences were found in Subscale 1 (Pearson correlation r = 0.389; p = 0.249). On the other hand, statistically significant differences were observed in Subscale 2 (r = 0.383; p = 0.031), implying that older parents had more negative feelings towards humanoid robots. Finally, no statistically significant differences were found in the level of social acceptance of humanoid robots between older and younger parents (Subscale 3; Pearson correlation r = 0.032; p = 0.861).

Discussion

The modern way of life and environmental influences during childhood and adolescence have led to the growing prevalence of health conditions that can be successfully resolved through (re)habilitation as a therapeutic mode. In the present study, robot-assisted therapy, including corrective exercises, was provided to children and adolescents with poor posture. One of the most important obstacles to the therapy success in this population is lack of motivation. Use of robots for medical purposes has opened a new era in this field, requiring a multidisciplinary approach [7, 9, 14].

No matter how advanced the technology, its true potential can only be ascertained by its adoption in everyday life, by making it accessible, and by determining people's attitudes towards its use. In this study, we aimed to establish the utility of humanoid robots in pediatric (re)habilitation by seeking input from parents whose children took part in the treatment involving a humanoid robot MARKO. As the majority of study participants were women, it may be assumed that mothers tend to be more involved in corrective exercise programs.

At present, there is paucity of research on attitudes and acceptance of humanoid robots. The youngest parent in our study was 27 years of age, while the oldest was 67 years old, with the average age of 41. Some of the prior studies on this topic, on the other hand, have been focused on students [15].

Data for this study were obtained via the FSQ. Other similar investigations used online surveys to

obtain participant input. Social and individual acceptance of new technologies is an increasingly relevant research domain. In particular, it is important to establish how new technologies are incorporated in everyday life, and if there are differences in attitudes with respect to gender, age, educational attainment, and cultural background. The FSQ is one of the instruments that can be used to assess the degree of humanoid robot acceptance [15, 16].

The article published by Kaplan was among the first to describe the Frankenstein syndrome, while also providing reasons for greater acceptance of humanoid robots in Japan compared to Western countries. As Japan is the world leader in the development of humanoid robots, its robotics culture is also most advanced [1, 17]. As mentioned above, there are hardly any researches in our area dealing with the attitudes and acceptance of humanoid robots. The results of our research show that parents have a neutral attitude towards humanoid robots and that their expectations of humanoid robots are positive.

To facilitate interpretation of findings yielded by the questionnaire, the items were grouped into three subscales, in line with the approach adopted by Nomura et al. in their investigation of human attitudes [18–20].

Results pertaining to Subscale 1 indicate that parents have neutral attitude towards humanoid robots, while those in Subscale 2 suggest that their expectations of humanoid robots are positive, and Subscale 3 findings show that parents have no negative feelings towards humanoid robots on the religious and psychosocial levels.

As expected, less educated parents have shown more negative feelings towards humanoid robots, as insufficient understanding can induce mistrust in patients, i.e., parents, due to anxiety and difficulty in accepting humanoid robots.

Social acceptance of humanoid robots is affected by a wide range of factors, including gender, age and cultural background [1, 14, 19]. Kaplan, for example, demonstrated the influence of culture, as his findings indicated that Japanese are "technology lovers" unlike their Western counterparts [1]. The results of our research show that there is no difference in the acceptance of humanoid robots with respect to gender, but that there is a difference with respect to age, that is, older parents have more negative feelings towards humanoid robots.

Some authors have also examined the effects of age on the degree of acceptance of humanoid robots. For example, Nomura et al. found that older individuals tend to have more positive expectations from practical application of this technology [19].

Bartneck et al, on the other hand, noted that gender was not the influential factor in the participants' attitudes towards humanoid robots, but found that nationality exerted significant effect on the degree of acceptance, whereby respondents from Netherlands and China exhibited lower degree of social acceptance of humanoid robots compared to Japanese participants [17].

In 2001, Syrdal et al. reported no statistically significant differences in attitudes of men and women from Japan and Great Britain, while age emerged as an influential factor. However, it is worth noting that the average respondent age in Japan was 24, while it was 29 in the sample from the United Kongdom [15, 20].

One of the main obstacles to robot use is fear of dehumanization, i.e., apprehension related to robots taking over human roles. Robots can be humanized in our society by modifying their visual appearance and interaction mode, which must be as human-like as possible, as well as by promoting their role as collaborators and friends [12, 21].

Conclusion

The findings of this investigation showed that parental attitudes towards humanoid robots and robot-assisted therapy are positive, and that most of them are informed of the latest technological advances through media. In our society, parents have neutral attitudes and positive expectations of humanoid robots and have accepted them socially, in the sense that they showed no negative feelings on religious or psychosocial levels. These attitudes are primarily influenced by educational attainment, as less educated parents were more apprehensive of technology, whereas no gender-related differences were observed. Parental attitudes are also affected by age, whereby older parents tend to be more anxious.

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PROFESSIONAL ARTICLES
STRUČNI ČLANCI

POTENTIALS OF THREE DIMENSIONAL PRINTING IN RADIOLOGY – A CASE OF A KNEE INJURY

MOGUĆNOSTI PRIMENE TRODIMENZIONALNIH ŠTAMPAČA U RADIOLOGIJI – SLUČAJ POVREDE KOLENA

Mirela JUKOVIĆ[1,2], Ivana STOJIĆ[1] and Viktor TILL[1,2]

Summary

Introduction. Images of computed tomography, computed tomography angiography, ultrasonography, magnetic resonance imaging, positron emission tomography are usually stored in Digital Imaging and Communication in Medicine standard formats, which can be used later for processing and making three dimensional models and objects. The three dimensional printing has become increasingly popular in various fields of medicine. The purpose of this article was to present the workflow for three dimensional printing of medical models and to point out potential significance of three dimensional model printing in clinical practice and in training of medical students and residents. This type of approach may open new perspectives in communication and interaction between clinicians, radiologists and patients, in order to achieve better treatment results. **Material and Methods.** Images of an injured knee in digital imaging and communication in medicine format were used for creation of a three dimensional printed model. Digital imaging and communication in medicine files were without sensitive information on the patient using three dimensional Slicer software, then processed with embodi three dimensional cloud service, further prepared with MeshLab and printed on a Ultimaker 2 printer. **Results and Discussion.** A three dimensional model of an injured knee was printed and presented. The model was used for the evaluation of tibial fractures. It may be shown to the patient and also to the surgeon in order to be more specific about the treatment procedure. Application of three dimensional printing in medicine was discussed. **Conclusion.** Medical three dimensional printing is likely to play a more important role in the clinical practice, not only for surgical planning, but also in the education of students and residents in different medical branches. This three dimensional plastic model of an injured knee may serve as a good example of the potentials of the three dimensional printing technology in medicine.
Key words: Printing, Three-Dimensional; Radiology; Knee Joint; Models, Educational; Education, Medical

Sažetak

Uvod. Slike dobijene pomoću kompjuterizovane tomografije, kompjuterizovane tomografske angiografije, ultrasonografije, magnetne rezonancije, pozitron-emisione tomografije obično se nalaze u formi koja prati *Digital Imaging and Communication in Medicine* standard i koje se mogu upotrebiti za kreiranje trodimenzionalnih modela i objekata. U različitim oblastima medicine trodimenzionalna štampa postaje sve popularnija. Svrha ovog rada bila je da se prikaže tok izrade štampanog modela i potencijalni značaj štampanja trodimenzionalnih modela u kliničkoj praksi i edukaciji studenata i specijalizanata. Ovakav pristup može otvoriti nove kanale u komunikaciji i interakciji između kliničara, radiologa i pacijenata, sa ciljem ostvarenja boljih rezultata lečenja. **Materijal i metode.** Slike fraktura zgloba kolena skladištene u formatu *Digital Imaging and Communication in Medicine* su korištene za kreiranje trodimenzionalnih modela za štampu. Iz fajlova *Digital Imaging and Communication in Medicine* su uklonjeni osetljivi podaci o pacijentu pomoću trodimenzionalnih *Slicer* softvera, dalje obrađeni pomoću internet servisa *embodi3d*, programa *MeshLab* i odštampane pomoću *Ultimaker 2* štampača. **Rezultati i diskusija.** Prikazan je trodimenzionalni model povređenog kolena. Model je korišćen za evaluaciju frakture tibije. On se može prikazati pacijentu, kao i hirurgu da bi se procenio dalji tok lečenja. Diskutovana je primena trodimenzionalne štampe u medicini. **Zaključak.** Očekuje se da primena trodimenzionalne štampe u medicini postane značajnija u kliničkoj praksi, ne samo u vezi sa planiranjem hirurških zahvata, već i u obrazovnom procesu studenata i specijalizanata u različitim medicinskim granama. Ovaj trodimenzionalni plastični model povređenog kolena može poslužiti kao dobar primer potencijala tehnologije trodimenzionalne štampe u medicini.
Ključne reči: 3D printing; radiologija; zglob kolena; edukativni model; medicinska edukacija

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Abbreviations
3D   – three dimensional
CT   – computed tomography
CTA   – computed tomography angiography
MRI   – magnetic resonance imaging
DICOM   – Digital Imaging and Communication in Medicine
2D   – two dimensional
STL   – standard tessellation language; stereolithography

Introduction

Three dimensional printing

Three dimensional (3D) printing, also known as rapid prototyping, allows creation of three dimensional objects using computer aided design information [1]. It refers to manufacturing technologies that produce physical models from information stored in a digital format [2]. It is an emerging discipline with potential application in various fields of medicine and biomedicine. Radiology is a discipline of images where information on patients, their pathologies, imaging data, and often interventional procedures encounter, so implementation of 3D printing and its technologies may be reasonable. Possibilities for applications of 3D models in healthcare are diverse, from preoperative planning, medical implant manufacturing, intraoperative guidance to education of students, physicians and patients education. This process was created in 1986, for use in architecture and manufacturing, called additive manufacturing or rapid prototyping. Medical 3D printing is a new, innovative method that converts two-dimensional (2D) scans from Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) into 3D objects, physically tangible, made of specific materials [3].

Three dimensional model creation

The process of 3D model creation is divided into three stages: image acquisition, image post-processing and 3D printing. The 3D printing of organs or some part of the organ starts from images stored in Digital Imaging and Communication in Medicine (DICOM) format. The acquisition of images can be performed by CT, CT angiography (CTA), MRI, Ultrasonography or Positron Emission Tomography (PET). The DICOM images are then transferred to Standard Tessellation Language, Standard Triangle Language or stereolithography (STL) format (or 3D file format) which is suitable and appropriate for 3D printers. Image post-processing allows segmentation of tissues using region of interest. Segmentation tools could be volume rendering technique, minimal intensity projection, and multiplanar reconstruction. Technically, DICOM stored 2D images are transformed into 3D models written in standard STL format. Those models are subjected to further manual editing and arranging which leads to the final shape of structure. Determination of the layers, temperature, color, tool path and printing speed are important for printing. The code that the slicer makes is sent to the 3D printer for the final model [2, 3].

Figure 1. Preview of the injured knee reconstruction using RadiAnt Digital Imaging and Communication in Medicine Viewer
Slika 1. *Prikaz rekonstrukcije zgloba kolena korišćenje RadiAnt Digital Imaging and Communication in Medicine Viewer-a*

The purpose of this article was to present the 3D printing workflow and to point out potential significance of 3D model printing in clinical practice and in medical training of students and residents.

Material and Methods

The 3D printing uses computer models to create 3D objects made of specific material layers that join together using computer aided design (CAD) and form the final shape and figure. This type of object is supposed to be printed. Then the model is exported as a STL format file, imported into slicing software and finally printed on a 3D printer.

Figure 2. 3D model created using the online service from embodi3d
Slika 2. *Trodimenzionalni model napravljen pomoću online servisa embodi3d*
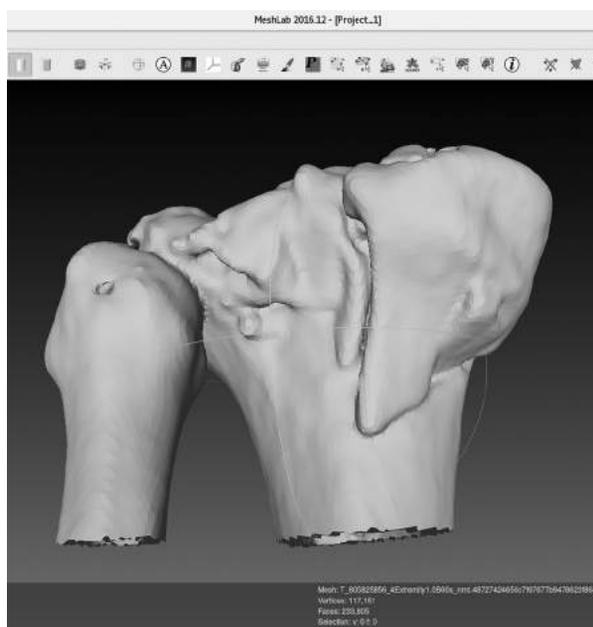
Figure 3. Region of interest segmented using MeshLab
Slika 3. Regija od interesa koja je segmentisana sa Mesh-Lab softverom

Images of an injured knee stored in DICOM format **(Figure 1)** were used for creation of a 3D printing model. The DICOM files were converted into nearly raw raster data format for the purpose of removing sensitive patient information (so-called anonymization process) using a 3D Slicer software, then processed with cloud service embodi3D to get a STL format **(Figure 2)** and finally the region of interest was segmented with MeshLab **(Figure 3)**. The model was printed on a Ultimaker 2 (https://ultimaker.com/) using polylactic acid (PLA) a thermoplastic polymer derived from renewable resources, such as corn starch or sugar cane. The printing process lasted 18 hours, and the injured knee mod-

Figure 4. 3D anatomical model printed using STL technology
Slika 4. Trodimenzionalni anatomski i štampani model dobijen tehnikom stereolitografije

el outsourcing the printing presented in this study cost around $ 35.

Results and Discussion

Potentials of 3D printing in the Radiology Center (Clinical Center of Vojvodina)
The 3D model of an injured knee was printed **(Figure 4)**. The model (size: 98.2 x 76.6 x 86.6 mm) was used for assessment of fractures among the clinicians. It was possible to show it to the patient for consent and decision making about treatment and also to the surgeon in order to be more specific about the possibilities of treatment in the future.

In our Radiology Center, routine practice has included making numerous 3D reconstructions as digital files using computer softwares, as images on films or prints on paper. It offers possibilities for presentation of different anatomical variations of the organs or pathology. This type of 3D reconstruction does not demonstrate the deep sensory feeling of anatomy and relationships with other structures in comparison with the real 3D model. It appears that 3D model may be a useful tool that gives a new dimension of touch and offers better understanding of complex relations between structures [4]. Application of currently available software at the Clinical Centre of Vojvodina (Carestream and Syngo.via) and 3D printing platforms may allow instant utilization of created 3D reconstructions and production of printed 3D models.

3D printing history
The 3D printer was invented in the beginning of the 1980s by Charles Hull, an engineer who worked on producing plastic devices from photopolymers. Just a few years later, in 1986, he established STL and developed the first commercial 3D printer - SLA-125. Eyewear frames were the first to be printed using 3D technology. For many years, it was used in manufacturing industry in producing product samples, such as airplane and car prototypes, in architecture for making structural reproductions, in government defense etc [5, 6]. Today, 3D printing is of great importance in clinical practice and experimental research [7].

3D printing in medicine
The 3D printing has been applied in medicine since the early 2000s and according to the available scientific reports it accelerated in the 2010s [2]. Today, 3D printing is widespread in maxillofacial surgery, orthopedics, plastic and vascular surgery, neurosurgery, cardiovascular interventions, in radiation oncology and could also be implemented in the interventional radiology using CTA data [8]. Most often, it is used in maxillofacial surgery for head and neck pathology (making prostheses, custom-made implants, etc.) [9]. A 3D printing model can be used in thoracic surgery as well, in planning of thoracoscopic procedures in patients with rare

anomalies or to show relations between a tumor and nearby structures. It was even used for a newborn baby with tracheomalacia to make a customized airway splint [10]. It can also be used in spine surgery for vertebral stabilization by creating 3D printing guides for optimal and precise hole drilling [11]. This process may be technically very challenging and the model helps surgery residents improve their skills and the outcome. Previous studies of 3D printed endoprostheses for palliative care of 16 patients with metastases of the humerus and ulna showed good outcomes in almost 70% of patients [12]. In the field of cardiac surgery, 3D models, based on cardiac CT, MRI and CTA, for visualization of different congenital heart problems, were applied, which played a significant role in the preoperative treatment, providing better understanding of the health condition of patients and education of students [13]. Also, a 3D printed model was used for rehearsal of planned myectomy in patients with hypertrophic cardiomyopathy [14]. The 3D printing has an important role in neurosurgery, in preoperative preparation and visualization of tissue variations and anomalies, as well as in many other surgical specialties. Patient-specific custom cutting guides are up to date as well as creation of patient-specific implants and bioprinting. Bioprinting is definitely the most impressive step in the 3D printing [15]. Its main aim is engineering functional healthy organs, but making an organ for transplantation, like liver for example, has not yet been done.

Costs, benefits and opportunities
Three dimensional printers are being used more and more, as their price is decreasing every day. For example, some basic 3D printer models cost around US $300, but sophisticated ones may cost up to US $ 1.000.000 [3]. Models made for medical purposes cost from US $50 to 2.500 [16]. The main benefit of 3D printing is a relatively low cost and a great number of possible applications in medicine [17]. Apart from using them for better comprehension of medical problems, additional tools for MRI diagnostic can be printed as well [18]. By using this process, better surgical outcomes and less time for surgical procedures can be expected. The radiologists may play an important role in reading the scans and converting them into 3D models [2]. This all requires a great involvement and collaboration between radiologists and physicians. The 3D printed models may also be used in training medical students and residents [19].

Conclusion

It is expected that three dimensional printing in medicine will play an important role in clinical practice, not only in surgery planning and therapy, but also in education of medical students and residents, as well as in simulation-based training in different medical branches. The three dimensional plastic model of an injured knee produced for the purpose of this study may serve as a good example on the potentials of this technology. Making three dimensional objects is not very demanding, and in the future, radiologists should be trained for implementation of three dimensional printing in complicated cases in the best interest of treatment and optimal recovery of patients.

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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF KLEBSIELLA PNEUMONIAE IN THE PERIOD 2013 - 2017

ISPITIVANJE OSETLJIVOSTI KLEBSIELLA PNEUMONIAE NA ANTIMIKROBNE LEKOVE U PERIODU 2013–2017. GODINE

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Summary

Introduction. Klebsiella pneumoniae is a Gram-negative non-motile, encapsulated bacterium. It is a significant opportunistic pathogen, especially in hospital conditions, where most multi-drug-resistant strains are present. The aim of this study was to determine the incidence of hospital infections caused by Klebsiella pneumoniae and the incidence of antimicrobial resistance of Klebsiella pneumoniae strains, routinely isolated from patients at the Clinical of Infectious Diseases of the Clinical Center of Vojvodina, in the period from January 1, 2013 to December 31, 2017. **Material and Methods.** A retrospective study included 1.647 patients with a diagnosis of infectious diseases of bacterial etiology treated at the Clinic of Infectious Diseases of the Clinical Center of Vojvodina. The analysis of primo-isolates from patient material (blood, urine, cerebrospinal fluid, wound/decubitus and throat cultures) was performed to examine the incidence of infections caused by Klebsiella pneumoniae and its antimicrobial resistance to antibiotics. **Results.** During the five-year study period, 93 primo-isolates of Klebsiella pneumoniae were isolated from urine cultures 52/272 (19.1%), throat swabs 13/108 (12%), wound swabs 12/120 (10.0%), blood cultures 13/285 (4.6%) and one isolate from the liver 1/47 (2.1%). Klebsiella pneumoniae isolates showed the highest sensitivity to tigecycline and colistin (100%). During the study, Klebsiella pneumoniae showed resistance to carbapenem in up to 100%, depending on isolates and the year of study. **Conclusion.** Klebsiella pneumoniae is a common cause of nosocomial infections. It is sensitive only to tigecycline and colistin (100%), and its resistance to carbapenems and other antibiotics is a major health concern.
Key words: Klebsiella pneumoniae; Klebsiella Infections; Drug Resistance; Anti-Bacterial Agents; Cross Infection; Microbial Sensitivity Tests; Tigecycline; Colistin; Carbapenems

Sažetak

Uvod. *Klebsiella pneumoniae* je Gram-negativna bakterija, nepokretna sa izraženom kapsulom. Značajan je uzročnik oportunističkih infekcija naročito u bolničkim uslovima u kojima su najčešće prisutni multirezistentni sojevi. Cilj rada je utvrđivanje učestalosti bolničkih infekcija izazvanih bakterijom *Klebsiella pneumoniae* i prisustva rezistencije na antimikrobne lekove kod sojeva *Klebsiella pneumoniae*, izolovanih iz bolesničkog materijala rutinski uzorkovanog na Klinici za infektivne bolesti Kliničkog centra Vojvodine u periodu od 1. 1. 2013. do 31. 12. 2017. godine. **Materijal i metode.** Retrospektivna studija je obuhvatila 1.647 pacijenata sa dijagnozom infektivnih bolesti bakterijske etiologije lečenih na Klinici za infektivne bolesti Kliničkog centra Vojvodine. Analizom primoizolata izolovanih iz bolesničkog materijala (hemokulture, urinokulture, kulture likvora, bris rane/dekubitusa i bris grla) pratili smo učestalost infekcija izazvanih bakterijom *Klebsiella pneumoniae* i njiovu osetljivost na antimikrobne lekove. **Rezultati.** Tokom petogodišnjeg ispitivanja izolovano je 93 primoizolata ove bakterije − iz urinokultura 52/272 (19,1%), iz brisa grla 13/108 (12%), iz brisa rane/dekubitusa 12/120 (10,0%), iz hemokultura 13/285 (4,6%) i jedan izolat iz likvora 1/47 (2,1%). Izolati *Klebsiella pneumoniae* bili su najosetljiviji na tigeciklin i kolistin (100%). Tokom svih godina istraživanja prisutna je pojava rezistencije na karbapeneme, u zavisnosti od izolata i godine istraživanja, koja je iznosila i do 100%. **Zaključak.** *Klebsiella pneumoniae* je značajan uzročnik infekcija kod hospitalizovanih pacijenata. Njen izolati bili su osetljivi jedino na tigeciklin i kolistin (100%), a zabrinjavajuća je pojava rezistencije na karbapeneme i ostale ispitivane grupe antibiotika. **Ključne reči:** Klebsiela pneumonije; infekcije Klebsielom; otpornost na lekove; antibiotici; bolnička infekcija; testovi osetljivosti na mikrobe; tigecikllin; kolistin; karbapenemi

Introduction

Antibiotic resistance continues to be one of the most serious public health issues, which is on the rise in all parts of the world. It presents a serious

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threat to the effective treatment of infectious diseases, and also to progress in medicine and health in general [1]. The World Health Organization (WHO) defines antimicrobial resistance as the resistance of micro-organisms to drugs that have previously been effective. Antimicrobial resistance is a broader term, which includes resistance to drugs used in the treatment of infections caused by other micro-organisms such as viruses, fungi and parasites [1]. According to the European Center for Disease Prevention and Control (ECDC), antimicrobial resistance is the ability of the micro-organism to become resistant to one or more antimicrobial agents used in therapy or prophylaxis. Multi-resistant bacteria (MRB) is the term used when bacterial strain is resistant to three or more groups of antibiotics [2].

Klebsiella is a genus of Gram-negative, rod shaped, non-motile, encapsulated bacteria. The species of Klebsiella can be found singly or in pairs, diplobacillus, with large polysaccharide capsule, which are different from most other species of the Enterobacteriaceae family. The capsule, a polysaccharide matrix which covers the cell, is the most important virulence factor. Fimbriae also contribute to virulence, allowing adherence to host epithelial cells and protect the bacteria from phagocytosis. The ability to produce aerobactin, that provides iron supply to bacteria necessary for growth and reproduction, is in strong correlation with virulence. The presence of urease is associated with the development

of urinary tract infections and leads to the formation of ammonia that damages the urinary tract epithelium and alkalizes the urine [3].

The representatives of this genus are widespread in nature; they are found in the soil, as well as in the intestinal tract of humans and animals. They are a major cause of opportunistic infections, especially in hospital environment, where they commonly become multi-resistant. Klebsiella related diseases are most common in urology departments, in intensive care units, and in newborns. Intestinal and respiratory tracts of hospitalized patients and hospital staff are the main reservoirs of infection, and they are most commonly transmitted through the medical workers' hands, rarely contaminated medical equipment and liquids [3].

In children and adults over 50 years of age, Klebsiella pneumoniae causes severe infections of the respiratory system, such as pneumonia (lobar pneumonia, bronchopneumonia) and lung abscesses. From the respiratory tract, infections can spread to the mucous membrane of paranasal sinuses, but cases of meningitis and septicemia are also reported. In children, it causes enteritis and enterocolitis, the symptoms of which resemble dysentery. It also causes urinary infections that are characterized by severe, uncertain response to antibiotics and frequent relapses.

Carbapenems are broad spectrum beta-lactam antibiotics, traditionally used as the first line therapy of the most serious infections [4]. When they appeared, in the eighties of the last century, they were the first-line drugs for the treatment of infections caused by Gram-negative agents and they presented a new possibility in the treatment of severe infections [4]. The clinical significance of carbapenemase enzyme is reflected in its ability to hydrolyze carbap-

Table 1. Antimicrobial susceptibility of Klebsiella pneumoniae isolated from blood

Tabela 1. Osetljivost izolata Klebsiella pneumoniae na antimikrobne lekove iz hemokultura

Antibiotic/*Antibiotik*	Klebsiella penumoniae/*Klebsiella pneumoniae*									
	2014		2015		2016		2017		Total/*Ukupno*	
	S	R	S	R	S	R	S	R	S	R
	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)
Ampicillin/*Ampicilin*	0 (0.0)	4 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	5 (100.0)	0 (0.0)	13 (100.0)
Amoxicillin and Clavulanic acid/*Amoksicilin i klavulanska kiselina*	0 (0.0)	4 (100.0)	2 (100.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	5 (100.0)	3 (23.0)	10 (77.0)
Piperacillin/*Piperacilin*	4 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	4 (80.0)	6 (46.0)	5 (38.0)
Imipenem/*Imipenem*	4 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	4 (80.0)	1 (20.0)	12 (92.0)	1 (7.7)
Meropenem/*Meropenem*	4 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	4 (80.0)	1 (20.0)	12 (92.0)	1 (7.7)
Gentamicin/*Gentamicin*	1 (25.0)	3 (75.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	2 (40.0)	3 (60.0)	4 (30.7)	7 (53.8)
Amikacin/*Amikacin*	4 (100.0)	0 (0.0)	1 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	5 (100.0)	6 (46.0)	6 (46.0)
Trimethoprim sulfamethoxazole/*Trimetroprini i sulfametoksazol*	2 (50.0)	2 (50.0)	2 (100.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	5 (100.0)	5 (38.0)	8 (61.0)
Ciprofloxacin/*Ciprofloksacin*	1 (25.0)	3 (75.0)	2 (100.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0,0)	5 (100.0)	4 (30.7)	9 (69.0)
Total/*Ukupno*	4	–	2	–	2	–	5	–	13	–

Legend: S - susceptible, R - resistant/*Legenda: S - osetljivo, R - rezistentno*

Table 2. Antimicrobial susceptibility of Klebsiella pneumoniae isolated from urine cultures
Tabela 2. *Osetljivost izolata Klebsiella pneumoniae na antimikrobne lekove iz urinokultura*

Antibiotic *Antibiotik*	Klebsiella penumoniae/*Klebsiella pneumoniae*											
	2013		2014		2015		2016		2017		Total/*Ukupno*	
	S	R	S	R	S	R	S	R	S	R	S	R
	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)
Ampicillin *Amipicilin*	0 (0.0)	5 (100.0)	0 (0.0)	4 (100.0)	1 (7.7)	12 (92.3)	0 (0.0)	8 (100.0)	0 (0.0)	22 (100.0)	1 (1.9)	51 (98.1)
Amoxicilln/ clavulanic acid *Amoksicilin i klavulanska kiselina*	0 (0.0)	5 (100.0)	0 (0.0)	4 (100.0)	2 (15.4)	11 (84.6)	4 (50.)	4 (50.)	4 (18.1)	18 (81.8)	10 (19.3)	42 (80.7)
Piperacillin/ tazobctam *Piperacilin i tazobaktam*	4 (80.0)	1 (20.0)	4 (100.0)	0 (0.0)	3 (23.0)	10 (77.0)	0 (0.0)	8 (100.0)	2 (9.1)	20 (90.9)	12 (23.0)	40 (77.0)
Imipenem *Imipenem*	4 (80.0)	1 (20.0)	4 (100.0)	0 (0.0)	13 (100.0)	0 (0.0)	7 (87.5)	1 (12.5)	10 (45.4)	12 (54.5)	38 (73.1)	14 (26.9)
Meropenem *Meropenem*	4 (80.0)	1 (20.0)	4 (100.0)	0 (0.0)	13 (100.0)	0 (0.0)	7 (87.5)	1 (12.5)	6 (27.2)	16 (72.7)	34 (65.4)	18 (34.6)
Gentamicin *Gentamicin*	1 (20.0) 0	4 (80.0)	2 (50.0)	2 (50.0)	3 (23.0)	10 (77.0)	7 (87.5)	1 (12.5)	1 (4.6)	21 (95.4)	4 (5.8)	48 (74.5)
Amikacin *Amikacin*	2 (40.0)	3 (60.0	3 (80.0)	1 (20.0)	9 (69.2)	4 (30.7)	6 (75.0)	2 (25.0)	7 (31.8)	15 (68.2)	27 (53.0)	25 (47.0)
Trimethoprim sulfamethoxazole/*Trimetroprim i sulfametoksazol*	2 (40.0)	3 (60.0)	2 (50.0)	2 (50.0)	1 (7.7)	12 (92.3)	0 (0.0)	8 (100.0)	1 (4.6)	21 (95.4)	6 (11,7)	46 (89.3)
Ciprofloxacin *Ciprofloksacin*	1 (20.0)	4 (80.0)	2 (50.0)	2 (50.0)	1 (7.7)	12 (92.3)	0 (0.0)	8 (100.0)	1 (4.6)	21 (95.4)	5 (7.8)	47 (92.2)
Tigecycline *Tigeciklin*	–	–	–	–	–	–	–	–	6 (100.0)	0 (0.0)	6 (100.0)	0 (0.0)
Colistin/*Kolistin*	–	–	–	–	–	–	–	–	6 (100.0)	0 (0,0)	6 (100.0)	0 (0.0)
Total/*Ukupno*	5	–	4	–	13	–	8	–	22	–	52	–

Legend: S - susceptible, R - resistant/*Legenda*: *S - osetljivo, R - rezistentno*

enems, which causes bacteria that produce them develop resistance to this group of beta-lactam antibiotics, as well as to most other members of the same group (penicillins, cephalosporins). Carbapenemase producing strains of Gram-negative bacteria are of great epidemiological significance, because spread of these beta-lactamases in hospitals and hospital isolates would present a serious global threat. The first carbapenem-resistant bacteria were described in the nineties of the last century, and their resistance was mediated by chromosomal coded beta-lactamases called carbapenemase [5]. Shortly after that, resistant strains of Klebsiella pneumoniae appeared in the United States, carrying transferable genes for carbapenemases located on plasmids. However, the resistance of these bacteria to this group of antibiotics remained sporadic until the beginning of the third millennium, when we became witnesses of the crisis due to the rapid spread of MRB of Klebsiella pneumoniae that produce carbapenemase encoded plasmids [6]. The main concern is that an in-creasing number of countries in Europe report a sudden spread of carbapenem-resistant Klebsiella pneumoniae (CRKP) strains. The global spread of the CRKP enterobacteria has become endemic in Mediterranean countries (Israel, Greece, Italy), South America (Colombia, Argentina and Brazil) and China [7–10]. The mortality reported among patients infected with these resistant bacteria is high, perhaps as a result of limited remaining antibiotic options (colistin and tigecycline). The introduction of tigecycline and colistin in the treatment of patients infected with these resistant strains has significantly improved the patients' survival [11].

Material and Methods

This retrospective study analyzed the medical records of 1.647 patients diagnosed with infectious diseases of bacterial etiology treated at the Clinic of Infectious Diseases of the Clinical Center of Vojvodina (CCV) in the period from January 1, 2013 to De-

cember 31, 2017. The data were obtained from medical histories and included patients with sepsis, urinary tract infections - complicated urinary tract infections, skin and subcutaneous tissue infections, respiratory infections and pneumonia. The antimicrobial resistance was established based on primo-isolates isolated from the patient material (blood, urine, cerebrospinal fluid, wound and throat cultures) that were routinely sampled at the Clinic of Infectious Diseases of the CCV in the indicated period.

The inclusion criteria were clinical picture of bacterial infection and laboratory findings in agreement with it. The results of isolation, identification and susceptibility to antimicrobial drugs were obtained from standard reports of the bacteriological examination of the submitted material. Isolation and identification was done using standard bacteriological techniques in the laboratories of the Center for microbiology at the Institute of Public Health of Vojvodina in Novi Sad. The distribution of the causative agents is shown through the absolute number of primo-isolates, as well as through the percentage of their occurrence in the calendar year when they were isolated. The sensitivity to antimicrobial drugs is expressed as the percentage of resistant and sensitive strains, and only primo-isolates were analyzed. The sensitivity to antimicrobial drugs was analyzed only if there were more than two bacteral isolates in one year. The strains with the intermediate sensitivity were classified into resistant (R). The strains that demonstrated resistance to three or more groups of antimicrobial drugs were considered multiresistant.

Results

During the five-year-study, a total of 3.073 isolates were examined, of which 847 isolates were positive and 93 primo-isolates of Klebsiella pneumoniae were isolated.

During the trial, a bacteriological examination of 1.332 blood samples for blood cultures was performed. A total of 285/1.332 (21.4%) primo-isolates were isolated from blood cultures. In 2013, there was a total of 46/474 (9.7%) primo-isolates, in 2014 62/172 (36%), in 2015, 49/205 (24%), in 2016, 62/222 (28 %), and in 2017 66/259 (25.4%) primo-isolates.

The most frequent causative agents from blood cultures, during the five-year study, were coagulase negative Staphylococcus spp found in 108/285 (38%), followed by Escherichia coli in 49/285 (17.1%), Staphylococcus aureus in 25/285 (8.7%), Klebsiella pneumoniae in 13/285 (4.6%), Acinetobacter spp and Streptococcus pneumoniae in 13/285 (5.6%), and Streptococcus viridians in 12/285 (4.2%).

Table 3. Antimicrobial susceptibility of Klebsiella pneumoniae isolated from throat swabs
Tabela 3. Osetljivost izolata Klebsiella pneumoniae na antimikrobne lekove iz brisa grla

Antibiotic *Antibiotik*	Klebsiella penumoniae/*Klebsiella pneumoniae*											
	2013		2014		2015		2016		2017		Total/*Ukupno*	
	S	R	S	R	S	R	S	R	S	R	S	R
	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)
Ampicillin *ampicilin*	0 (0.0)	3 (100.0)	0 (0.0)	5 (100.0)	0 (0.0)	4 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (100.0)
Amoxicillin and clavulanic acid *Amoksicilin i klavulanska kiselina*	1 (33.3)	2 (66.6)	1 (20.0)	4 (100.0)	1 (25.0)	3 (75.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	3 (23.0)	10 (77.0)
Piperacillin tazobactam/*Piperacilin i tazobaktam*	2 (66.6)	1 (33.3)	2 (40.0)	3 (60.0)	1 (25.0)	3 (75.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	5 (38.0)	8 (62.0)
Imipenem *Imipenem*	3 (100.0)	0 (0.0)	5 (100.0)	0 (0.0)	4 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (100.0)	0 (0,0)
Meropenem *Meropenem*	3 (100.0)	0 (0.0)	5 (100.0)	0 (0.0)	4 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (100.0)	0 (0.0)
Gentamicin *Gentamicin*	–	–	5 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	5 (32.0)	8 (62.0)
Amikacin *Amikacin*	3 (100.0)	0 (0.0)	5 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (69.3)	4 (30.7)
Trimethoprim sulfamethoxazol *Trimetroprini i sulfametoksazol*	2 (66.6)	1 (33.3)	2 (40.0)	3 (60.0)	0 (0.0)	4 (100.0)	0 (0.0)	1(100.0)	0 (0.0)	0 (0.0)	4 (30.7)	9 (69.3)
Ciprofloxacin *Ciprofloksacin*	1 (33.3)	2 (66.6)	2 (40.0)	3 (60.0)	0 (0.0)	4 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	3 (23.0)	10 (77.0)
Total/*Ukupno*	3	–	5	–	4	–	1	–	0	–	13	–

Legend: S - susceptible, R - resistant/*Legenda: S - osetljivo, R - rezistentno*

Table 4. Antimicrobial susceptibility of Klebsiella pneumoniae isolated from woud/decubitus swabs
Tabela 4. Osetljivost izolata Klebsiella pneumoniae na antimikrobne lekove iz brisa rana/dekubitusa

Antibiotic *Antibiotik*	Klebsiella penumoniae/*Klebsiella pneumoniae*											
	2013		2014		2015		2016		2017		Total/*Ukupno*	
	S	R	S	R	S	R	S	R	S	R	S	R
	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)	N/*Br* (%)
Ampicillin *Ampicilin*	0 (0.0)	3 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)	3 (100.0)	0 (0.0)	12 (100.0)
Amoxicillin and clavulanic acid *Amoksicilin i klavulanska kiselina*	0 (0.0)	3 (100.0)	0 (0.0)	2 (100.0)	1 (100.0)	0 (0.0)	1 (33.3)	2 (66.6)	3 (100.0)	0 (0.0)	5 (41.6)	7 (58.4)
Piperacillin tazobactam/*Piperacilin i tazobaktam*	0 (0.0)	3 (100.0)	0 (0.0)	2 (100.0)	1 (100.0)	0 (0.0)	1 (33.3)	2 (66.6)	3 (100.0)	0 (0.0)	5 (41.6)	7 (58.4)
Imipenem *Imipenem*	0 (0.0)	3 (100.0)	0(0.0)	2 (100.0)	1 (100.0)	0 (0.0)	2 (66.6)	1 (33.3)	3 (100.0)	0 (0.0)	6 (50.0)	6 (50.0)
Meropenem *Meropenem*	0 (0.0)	3 (100.0)	0 (0,0)	2 (100.0)	1 (100.0)	0 (0,0)	2 (66,6)	1 (33.3)	3 (100.0)	0 (0.0)	5 (41.6)	7 (58.4)
Gentamicin *Gentamicin*	1 (33.3)	2 (66.6)	2 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (33.3)	2 (66.6)	1 (33.3)	2 (66.6)	5 (41.6)	7 (58.4)
Amikacin *Amikacin*	2 (66.6)	1 (33.3)	2 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	2 (66.6)	1 (33.3)	1 (33.3)	2 (66.6)	8 (66.6)	4 (33.3)
Trimethoprim sulfamethoxazol *Trimetroprini i sulfametoksazol*	0 (0.0)	3 (100.0)	0 (0.0)	2 (100.0)	1 (100.0)	0 (0.0)	1 (33,3)	2 (66,6)	3 (100.0)	0 (0.0)	5 (41.6)	7 (58.4)
Ciprofloxacin *Ciprofloksacin*	0 (0.0)	3 (100.0)	1 (50.0)	1 (50.0)	1 (100.0)	0 (0.0)	1 (33.3)	2 (66.6)	3 (100.0)	0 (0.0)	6 (50.0)	6 (50.0)
Tigecycline *Tigeciklin*	–	–	–	–	–	–	1 (100.0)	0 (0.0)	–	–	1 (100.0)	0 (0.0)
Colistin/*Kolistin*	–	–	–	–	–	–	1 (100.0)	0 (0.0)	–	–	1 (100.0)	0 (00)
Total/*Ukupno*	3	–	2	–	1	–	3	–	3	–	12	–

Legend: S - susceptible, R - resistant/*Legenda: S - osetljivo, R - rezistentno*

Other causative agents accounted for less than 4%. Klebsiella pneumoniae was isolated in 1/46 (2.2%) in 2013, 4/62 (6.4%) in 2014, 2/49 (4.08%), in 2015, 3/62 (4.8%) in, 2016, and 3/66 (4.5%) in 2017.

During the trial, bacteriological examination of 1.175 urine cultures was performed. Out of the total number, 272/1.175 (23.2%) of primo-isolates were isolated. In 2013, there were 47/274 (17.1%), in 2014 38/195 (14.3%), in 2015 68/218 (31.2%), in 2016 45/213 (21.1%), and in 2017 74/275 (27%). During the five-year trial, Escherichia coli was isolated from 69/272 (25.3%) urine cultures. The following are Klebsiella pneumoniae with 52/272 (19.1%), Enterobacter spp 50/272 (18.3%), Proteus mirabilis 23/272 (8.4%), Pseudomonas aeruginosa 22/272 (8.0%), and Acinetobacter spp 6/272 (2.2%). Other causative agents accounted for less than 2%. The annual frequency of Klebsiella pneumoniae positive isolates was: 5/47 (10.6%) in 2013, 4/38 (10.5%) in 2014, 13/68 (19.1%), in 2015, 8/45 (17.7%) in 2016, and 22/74 (29.7%) in 2017.

The test results of 215 samples of cerebrospinal fluid showed that 47/215 (21.8%) samples were positive for microorganisms. In 2013, there were 10/69 (14.5%) primo-isolates, in 2014 there were 13/43 (30.2%), in 2015 6/22 (27.3%), in 2016 9/53 (17%), and in 2017 9/28 (32.1%). The most commonly isolated microorganism from the cerebrospinal fluid was Streptococcus pneumoniae with 15/47 (31.9%). The following were coagulase-negative Staphylococcus spp with 14/47 (29.7%), Listeria monocytogenes and Neisseria meningitidis with 4/47 (8.5%), Acinetobacter spp and Streprococcus viridans, which were isolated with the same frequency of 3/47 (6.3%). Klebsiella pneumoniae was isolated in one case, 1/47 (2.1%). In 2013, 1/10 (10%) Klebsiella pneumoniae positive isolate was isolated from cerebrospinal fluid. In all the other years, there were no positive isolates in cerebrospinal fluid.

In the five-year study, bacteriological examination of 146 wound/decubitus swabs was performed. From the examined wound/decubitus swabs, 120/146 (82.2%) primo-isolates were isolated. In 2013, there were 34/47 (72.3%), in 2014, 35/38 (92.1%), in 2015, 14/15 (9303%), in 2016, 17/20 (85%) primo-isolates, and in 2017 20/26 (77%). The most frequently iso-

lated microorganisms in the wound swabs were Acinetobacter spp with 30/120 (25%), Staphylococcus aureus with 30/120 (25%) and Pseudomonas aeruginosa with 26/120 (21.6%). They were followed by Proteus mirabilis 14/120 (11.6%), Enterobacter spp 12/120 (10%), Klebsiella pneumoniae 12/120 (10.0%) and Enterococcus spp with 8/120 (6.6%). The annual incidence of Klebsiella pneumoniae positive isolates was: 3/34 (8.8%) in 2013, 2/35 (5.7%) in 2014, 1/14 (7.1%) in 2015, 3/17 (17.6%) in 2016, and 3/20 (15%) in 2017.

In the same period, bacteriological examination of 173 throat swabs was performed. From the examined throat swabs, 104/173 (60.1%) primo-isolates were isolated. In 2013, 37/42 (88%) were isolated, 35/40 (87.2%) in 2014, 13/30 (43.3%), 16/33 (48.4%) in 2016, and 3/28 (10.7%) in 2017. Staphylococcus aureus, with 50/104 (46.2%), was the most commonly isolated as the causative agent of infection. Then followed Acinetobacter spp with 14/104 (13%), Klebsiella pneumoniae with 13/104 (12%), Streptococcus pyogenes with 9/108 (8.3%), Enterobacter spp with 8/108 (7.4%), and Pseudomonas aeruginosa with 5/104 (4.6%). The annual frequency of Klebsiella pneumoniae positive isolates was: 3/37 (8.1%) in 2013, 5/35 (14.2%) in 2014, 4/13 (30.7%) in 2015, 1/16 (6,25%) in 2016, and 0/3 (0%) in 2017.

Klebsiella pneumoniae blood culture isolates showed resistance to all antimicrobial drugs, except imipenem and meropenem until 2017, when resistance to these antibiotics was present in one isolate, 1 (20%). The sensitivity of Klebsiella pneumoniae to carbapenems was 100% from 2013 to 2016 **(Table 1)**.

The resistance of Klebsiella pneumoniae isolates from urine cultures during 2014 and 2015 was established to all investigated antibiotics, except imipenem and meropenem. During 2016, resistance to carbapenem was found, and in 2017 there was a significant resistance of this bacteria to imipenem 12 (54.5%), and to meropenem 16 (72.7%). In 2017, susceptibility to tigecycline was found in six isolates, while the isolates were resistant to all the other tested antibiotics **(Table 2)**.

The antimicrobial susceptibility of Klebsiella pneumoniae isolates from throat swabs showed a 100% sensitivity to carbapenems (imipenem and meropenem). Its 100% resistance to aminoglycoside antibiotics (gentamicin) was observed in 2015 and 2016, as well as resistance to trimethoprime/sulfamethoxazole in 1 isolate (33.3%) in 2013, 3 isolates (60%) in 2014, and 4 isolates (100%) in 2015 **(Table 3)**.

The sensitivity of isolates of Klebsiella pneumoniae to antimicrobials from the wound/decubitus swabs showed 100% resistance to carbapenems (meropenem and imipenem) in 2013 and 2014, so that in 2017 the resistance in 3 isolates would be 0%. The sensitivity of Klebsiella pneumoniae to piperacillin/tazobactam was 0% during 2013 and 2014, and in 2017 it was 100%. The limitation of this research is a small number of isolates. In 2016, susceptibility to tigecycline and colistin was shown in one isolate, while it was resistant to all the other tested antibiotics **(Table 4)**.

Discussion

Klebsiella pneumoniae is one of the most common causes of urinary tract infections [12, 13]. The results of our five-year research showed that this bacterium is most often isolated from urine cultures, accounting for 19.1%. The number of primo-isolates of Klebsiella pneumoniae from urine cultures has significantly increased, in 2013 it accounted for 10.6%, and in 2017 for 29.7%. This is an indication that urinary tract infections caused by this bacterium have been on the rise. The African studies show similar results, so in Ethiopia, urinary infections account for 20%, Cameroon 18.5% and Marrakesh 22% [14, 15]. At the second and third place are primo-isolates of Klebsiella pneumoniae accounting for 12% in throat swabs and for 10% in the wound/decubitus swabs. In regard to the causative agents of throat infections, the most common is primo-isolate of Staphylococcus aureus (46.2%), while primo-isolate of Klebsiella pneumoniae is ranked third, accounting for 12%. These data are in accordance with a study done at the Clinic of Infectious Diseases of the CCV from 2012 to 2014, where Klebsiella pneumoniae is in the third place (9.3%), while Staphylococcus aureus is the first accounting for 48.6%, and in the second place is Acinetobacter spp, accounting for 11.2% [16]. The wound/decubitus swabs showed that primo-isolate of Klebsiella pneumoniae is even more frequent, but it is only ranked sixth in frequency (10%). Acinetobacter spp was the most common cause of wound/decubitus (25%) infections. This shows that Klebsiella pneumoniae is a rare cause of throat, wound/decubitus infections, and that there is no significant increase in its incidence during the investigated period. The results of the United States studies show that Klebsiella pneumoniae is the fourth cause of infections accounting for 8% of infections, while the first is Staphylococcus aureus (16%) [17]. Klebsiella pneumoniae in our five-year study has proven to be a rare cause of sepsis, bacterial meningitis, and meningoencephalitis. However, Klebsiella pneumoniae is the most commonly isolated microorganism from the cerebrospinal fluid, and it was isolated from one sample in 2013, with an incidence of 2.1%. In regard to causative agents isolated from blood cultures, the most common isolates were Staphylococcus spp coagulase negative (38%), while Klebsiella pneumoniae occupies the fourth position in frequency (4.6%).

All primo-isolates of Klebsiella pneumoniae from blood cultures in the study are susceptible to imipenem and meropenem, but the sensitivity has decreased over the years from 100% during 2014, 2015, 2016 to 80% in 2017. Similarly, it has been noticed in studies performed at the territory od European Union that the antibiotic resistance is below 5% [18–20]. The highest antimicrobial resistance during our five-year study was found for ampicillin (100%), amoxicillin/clavulanic acid (77%), ciprofloxacin (69%) and trimethoprim/sulfamethoxazole (61%). Gentamicin, which belongs to the second gen-

eration of aminoglycosides, shows resistance in 54% of cases, while resistance to amikacin is 46%. A twelve year study from the United States shows an increase in resistance to previously mentioned antibiotics, most notably ciprofloxacin and piperacillin/tazobactam. In blood cultures, resistance to imipenem was 5.9%, while in our study it increased to 7.7%. The highest increase in resistance was recorded to ciprofloxacin from 18.9% to 69% and trimethoprim/sulfamethoxazole from 21% to 61%, as well as to gentamicin, where the increase in resistance was five times higher, from 10% to 50%, and to amikacin ten times higher, from 4.2% to 46% [21]. Studies conducted in Spain, from 2010 to 2014, indicate resistance to imipenem 1.7%, ciprofloxacin 20.1%, gentamicin 10.4% and amikacin 1.9%, which is significantly lower than in our study [22]. When we compare the results of our study with available data from the National Reference Laboratory, we can notice a slight increase in resistance to amixocillin/clavulanic acid from 92.8% in 2013. to 100% in 2014 in our study [23].

The largest number of primo-isolates of Klebsiella pneumoniae are from urine cultures. Over the years, we noted that the highest resistance is expressed in beta-lactam and sulphonamide, which is over 80%. The smallest resistance was found to imipenem and meropenem, but significant changes have been observed over the years, so in 2016, where the resistance was about 12%, there was a rapid increase in 2017, with resistance to imipenem of 54% and meropenem of 72%, as well as the number of primo-isolates of Klebsiella pneumoniae from 10.6% to 29.7%. In 2017, in six isolates of this bacterium from urine cultures, susceptibility to tigecycline was obtained, while all the other tested antibiotics were resistant. All this shows an increasing resistance of Klebsiella pneumoniae to antimicrobial drugs, as well as a significant increase in multi-resistant strains in 2017. According to data from 2013, resistance to carbapenems in the European Union was less than 5% [18], which does not coincide with our study where it was 20%. When we compare our results with the results of a study from the United States, our study showed a marked increase in ciprofloxacin resistance from 15% to 95%, and trimethoprim/sulfamethoxazole from 18.3% to 91%. There was an increase in resistance to imipenem from 3.8% to 30% [21]. The study conducted at the Clinic of Infectious Diseases of the CCV in the period from 2012 to 2014, showed no resistance to Klebsiella pneumoniae to carbapenems, except for one isolate in 2013 that was resistant [16]. In this study, resistance to trimethopime/sulfamethoxazole, a drug that has been widely used in general practice in urinary infections, is about 50%, while data from Korea indicate a significantly lower percentage of resistance to this antibiotic (18%) [23]. The high percentage of resistance to trimethoprim/sulfamethoxazole is most likely due to a large use of this drug in the treatment of urinary infections. The resistance data confirm that this antibiotic should no longer be used as the first line therapy in uncomplicated urinary infections [24]. Over the past 20 years, a wide range of antimicrobial drugs has been used in the treatment of urinary tract infections caused by Gram-negative bacteria in the world. However, these microorganisms have developed multiple mechanisms of resistance to antibiotics [25], as shown in our study.

Out of a total of 12 primo-isolates of Klebsiella pneumoniae, in the period from 2013 to 2017, 100% sensitivity to carbapenems (imipenem, meropenem) was observed, and the highest (100%) resistance to ampicillin. Amikacin also showed to be sensitive, while in 2017, gentamicin, a drug from the same group, was detected to be 100% resistant, which is contrary to the study of Ndip et al. which found that gentamicin was the only antibiotic sensitive in the treatment of respiratory infections [26]. At the end of the examined period (2016 and 2017), amoxicillin/clavulanic acid, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole developed 100% resistance, although in 2013 the sensitivity varied from 33.3 to 66.6%. Similar results were also observed in ciprofloxacin, from 33.3% in 2013, up to 100% resistant in 2017. In 1996, Hobson et al. reported that eradication of Klebsiella pneumoniae was not possible with ciprofloxacin, nor cephalosporins, not even the third generation, but exactly the opposite, they induced the development of resistance, which is in accordance with our findings [27].

The results obtained out of 12 primo-isolates of Klebsiella pneumoniae from the wound/decubitus swabs show sensitivity to imipenem and meropenem of 50%, unlike other antibiotics where the sensitivity is much higher, even up to 100%. The highest resistance was expressed to ampicillin (100%) and amoxicillin/clavulanic acid, piperacillin/tazobactam, trimethoprim/sulfamethoxazole (58%). In 2016, an antibiogram showed resistance to all tested antibiotics, except for tigecycline and colistin. The currently available drugs for the treatment of carbapenem-resistant Klebsiella pneumoniae are precisely colistin and tigecycline [28]. Their sensitivity was investigated in our study, and occurrence of carbapenems-resistant bacteria was noticed. In India, an analysis of Klebsiella pneumoniae wound isolates found a sensitivity of 7.4% for ampicillin, 90% for imipenem and 84% for meropenem, while amoxicillin/clavulanic acid was 17% and piperacillin/tazobactam 64%. The sensitivity to gentamicin was 37% and to amikacin 61%, while in our opinion, the sensitivity to gentamicin was 41.6% and to amikacin 66.6%, which is in accordance with our study. Similar results are obtained by comparing these two studies, so the sensitivity to ciprofloxacin was 44%, while in our study it was 50% [29].

Klebsiella pneumoniae was not isolated from cerebrospinal fluid, except in one isolate from 2013. This shows that this bacterium is a rare cause of bacterial meningitis and meningoencephalitis.

Conclusion

Klebsiella pneumoniae is a common cause of infections. The primo-isolates of this bacterium from urine cultures were found in 52/272 (19.1%), followed by throat swabs in 13/108 (12%), and wound/decubitus swabs in 12/120 (10.0%). These are followed by isolates

from blood cultures in 13/285 (4.6%), and cerebrospinal fluid isolates in 1/47 (2.1%). During 2013 and 2014, blood culture isolates of Klebsiella pneumoniae showed sensitivity to carbapenems in 100%, and in 2017, 1 isolate was resistant to these drugs. From 2014 to 2016, urine culture isolates of Klebsiella pneumoniae showed sensitivity to carbapenems in 100%, in 2016 1 isolate was resistant to carbapenems, whereas in 2017 the resistance increased significantly to imipenem in 54.5% and to meropenem in 72.7%. In 2017, the sensitivity to tigecycline was 100%. In all Klebsiella pneumoniae throat isolates, in the investigated five-year period, the sensitivity to carbapenems was 100%. Isolates of Klebsiella pneumoniae from the wound/decubitus swabs in 2013 and 2014, showed a 100% resistance to carbapenems, in 2015 one isolate and in 2017 in 3 isolates the sensitivity to carbapenems was 100%. There was also a 100% sensitivity to tigecycline and colistin. Continuous monitoring of the Klebsiella pneumoniae resistance and rational use of antibiotics can prevent further development of multiresistant strains.

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PSYCHOLOGICAL MEDICINE

PSIHOLOŠKA MEDICINA

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Summary

Introduction. It is known that it is possible to improve healthcare by paying more attention to psychological aspects of diagnosis and treatment of physical and mental conditions. The study and practice of such factors is called psychological medicine. The aim of this paper was to provide contemporary aspects of psychological medicine and to emphasize its contributions to clinical practice. **Material and Methods.** A review of the literature on psychological and psychosomatic medicine was performed using Medline and manual search. **Results**. The main goals of psychological medicine are comprehensive assessment of psychological factors affecting the etiology, course and outcome of illness, biopsychosocial consideration of patient care, as well as integration of specific interventions in prevention, treatment and rehabilitation of patients. We need to emphasize that due to clinical limitations, in terms of current separation of mental and physical healthcare, the treatment of many health conditions in which psychological factors are significant, is still insufficient. **Conclusion.** Because of its importance to patients' well-being, psychological and psychosomatic medicine should to be an integral part of clinicians' knowledge across all specialties.
Key words: Psychosomatic Medicine; Stress, Psychological; Medically Unexplained Symptoms; Life Change Events; Personality; Neurotransmitter Agents; Immunity; Mental Health

Sažetak

Uvod. Poznato je da se zdravstvena zaštita može unaprediti uzimanjem u obzir psiholoških aspekata u dijagnostici i tretmanu svih somatskih i mentalnih bolesti. Izučavanje i implementaciju ovih faktora nazivamo psihološkom medicinom. Cilj istraživanja je da predstavimo savremene aspekte psihološke medicine i da naglasimo njen doprinos kliničkoj praksi. **Materijal i metode.** Proučavana je literatura koja se odnosi na psihološku i psihosomatsku medicinu iz indeksne baze *Medline*. **Rezultati.** Glavni ciljevi psihološke medicine su sveobuhvatna procena psiholoških faktora koji utiču na etiologiju, tok i ishod bolesti, zatim biopsihosocijalno razmatranje pacijenta, kao i integrisanje specifičnih intervencija u okvir prevencije, tretmana i rehabilitacije bolesnika. Naglašavamo da je zbog kliničkih ograničenja u smislu aktuelno prisutne separacije zaštite mentalnog i telesnog zdravlja tretman mnogih zdravstvenih stanja u kojima su psihološki faktori signifikantni i dalje insuficijentan. **Zaključak.** Psihološka i psihosomatska medicina zbog svog značaja za dobrobit pacijenata moraju biti integralni deo inventara znanja lekara svih specijalnosti.
Ključne reči: psihosomatska medicina; psihološki stres; medicinski neobjašnjeni simptomi; životne promene; ličnost; neurotransmiteri; imunitet; mentalno zdravlje

Introduction

It is known that paying more attention to psychological factors in the assessment and treatment of medical conditions can improve the healthcare. The study and practice of these factors is often called psychological medicine. Modern science has recognized the need for the medical discipline which observes the patient with all its somatic, psychological and social characteristics [1]. The essence of psychological medicine dates back to the earliest periods of medicine, when it became clear that certain psychological factors may play an important role in the etiology and treatment of various medical conditions. The connection between medicine and psychology in the integrated treatment of main organic and mental disorders is a key achievement of the biopsychosocial approach to healthcare and medical research in the modern era [2]. This discipline appeared in the European literature in the 19th century, gradually freeing itself from the academic and experimental psychology, in order to focus on the patient and his condition. Despite the fact that a large number of studies point to the importance of psychological factors, especially in the etiology, course and outcome of various health disorders, these factors remain forgotten in the knowledge inventory, particularly in the clinical work of medical doctors of all specialties [3]. Modern medicine is advancing rapidly using innovative biological and technological knowledge, often neglecting psychological factors that sometimes play an important role in all its areas [4]. The importance of these factors is of utmost importance in chronic,

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Abbreviations

CRH　　　– corticotropin releasing hormone
ACTH　　– adrenocorticotropic hormone
CRF　　　– corticotropin releasing factor
HPA　　　– hypothalamic-pituitary-adrenal
IL　　　　– interleukin

young, old, and dying patients. As a result, literature findings suggest a significantly better prognosis if the treatment of somatic disorders includes psychological knowledge in terms of treatment adherence, as well as prompt and adequate management of any existing comorbid mental problems [5]. These interventions improve the efficiency, safety and cost of healthcare in general [6].

Material and Methods

A review of the literature on psychological and psychosomatic medicine was performed using both Medline and manual searches. Classification of studies, in relation to their quality, was performed using the guideline for levels of evidence established by the American Academy of Neurology.

Results

Epidemiological studies have shown that up to 20% of the general population presents with psychosomatic, psychiatric, or psychological symptoms that need treatment [7]. The need for the development of psychological medicine arises from the fact that at least 25 – 30% of patients suffering from somatic diseases have comorbid depressive, anxiety, and somatoform or alcohol use disorder [8]. It is known that cardiovascular, neurological, malignant and endocrine diseases increase the risk of depression and other mental disorders [9]. On the other hand, studies suggest that about 30% of somatic symptoms cannot be explained by the underlying disease [10, 11]. However, the implementation of the principles of psychological medicine in general medicine does not automatically imply that all these patients are psychiatric patients.

The patient, not his disease, is and must remain the focus of medical science. This is stressed because it is known that progress in modern medicine led to medical procedures for the diagnosis and treatment of specific health conditions, but with deficiencies in the assessment of a patient's overall health status, with all his biological, psychological and social characteristics. Another important task of psychological medicine is directing the patient's energy and motivation to actively participate in the treatment, but also to prepare him for potential loss and isolation [5]. From the aspect of psychological medicine, health is not merely the absence of disease and the state of physical, mental and social well-being, as it is officially defined; it should include confrontation, reaction and adaptation strategies to cope with various changes and situations he constantly faces. Every disease affects the psychological, physical and social

balance of an individual by threatening to destroy the patient's functioning and quality of life.

The outdated model of disease implies that an external cause leads to physical damage and somatic symptoms, but it cannot explain the development of hypertension, cancer or a mental disorder. This is the reason why we believe that diseases are caused by multiple factors, including genetics, environmental factors, external biological factors, stress, adverse life events and lifestyle.

When it comes to the etiology of the disease, there are three principal factors:

1. Individual predisposition in terms of genetics and early environmental influences that produce biological and/or psychological vulnerability;

2. Precipitating factors – internal or external stressors and/or biological or chemical agents;

3. Disease supporting factors - physiological body changes, as well as psychological and social factors.

Besides that, there are two models of psychological medicine. The first is the medical model, which classifies the diseases based on a set of symptoms associated with corresponding anatomical and physiological changes in the body and which is based on the premise that health and disease are objective and measurable phenomena. The second model is the individual model and it deals more with the individual disease and less with the diagnostic criteria, but the emphasis is on the personal experience of the patient. Psychological medicine includes both models and takes advantage of the medical model which facilitates the understanding of disease due to medical aspects but takes into account the specific meaning of symptoms for a particular person, especially considering previous individual experiences in terms of disease and medical staff, current life situation, relations with the environment, emotional and adaptive abilities.

The relationship of stress and illness

It is believed that stress, as a negative emotional experience, is accompanied by biochemical, physiological, behavioral and cognitive changes in humans that can contribute to the occurrence, course and prognosis of certain physical and mental disorders [12].

We know that certain diseases may be caused mostly by unpleasant psychological factors, if there is a biological predisposition to the disease. Generally speaking, psychosomatic disorders could be basically explained as somatized anxiety. The aforementioned somatization is the mechanism by which the emotional content is transformed into the physical sphere. Therefore, chronic anxiety leads to changes in the body structure and psychosomatic diseases.

Stress and personality

Stress is in fact a highly personalized phenomenon which involves subjective perception and assessment. Even Hippocrates argued that it is far more important to know what person the disease has than what disease the person has. This implies that different types of personalities may develop

certain behaviors (determined by a variety of biological, psychological and social factors) which may lead to a disease. Certainly, it can be concluded that vulnerability to stress, and success in facing and overcoming it, depends on cognitive and affective personality traits, as well as personality organization and dominant defense mechanisms employed in stressful situations. Persons who are resistant to psychosomatic diseases are able to control themselves in stressful situations, they easily adapt to environmental changes, use social support and see stress as a challenge instead of a threat [13].

Life events
Various life events, in addition to the characteristics of a person exposed to their effects, can cause certain mental and somatic symptoms. In the mid-twentieth century, psychiatrists Holmes and Rahe developed the Social Readjustment Rating Scale for identifying stressful life events which consists of 43 life events associated with different intensity of stress for average individuals [14]. According to this scale, the most stressful events are death of a spouse, death of a child, divorce, and jail sentence, death of parents or friends, physical illness, marriage, job, retirement, illness of close relatives, pregnancy, childbirth, sexual problems, business problems, and financial problems.

Specific and non-specific stress factors
In addition to the negative influence of non-specific stressors, such as death of a spouse or divorce, some researchers suggested that persons with a specific psychological structure are prone to certain psychosomatic diseases. They found that competitiveness, stubbornness and aggressiveness were psychological characteristics connected with coronary diseases. Franz Alexander, an American psychoanalyst and founder of psychosomatic medicine, was a proponent of the theory that the specific unconscious conflicts are associated with specific psychosomatic diseases [15]. In this sense, he thought that peptic ulcer patients have a strong unmet need for dependence, and people with hypertension have hostile impulses about which they feel guilty.

Neurotransmitters and stress
Recent studies indicate that chronic stress can cause pathological changes of certain brain structures (e.g., reduction in hippocampal volume). It is assumed that psychosomatic illness has a strong connection with hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system disorders. Cerebral cortical centers react to adverse stimuli through the limbic system and lead to the activation of specific neuroendocrine systems. In response to stress, the hypothalamus secretes a corticotropin releasing hormone (CRH) which through the HPA axis and the secretion of pituitary adrenocorticotropic hormone (ACTH) stimulates the synthesis and release of steroids. Steroids have various effects on the body functions and can be briefly summed up as promoters of energy consumption and increased cardiovascular activity. This theory is supported by neuroimaging studies which have determined that anxiety provokes regional metabolic changes in the brain that can even be mapped. Stress activates the noradrenergic system of the brain and promotes the release of catecholamines from the autonomic nervous system. Stressors also activate the brain serotonergic system, resulting in its increased activity. It is believed that glucocorticoids enhance the overall activity of serotonin, and that there may be differences in the regulation of certain serotonin receptor subtypes. This is important for understanding the neurobiochemical changes in depression and anxiety disorders. There is also evidence that stress stimulates dopamine neurotransmission in the medial prefrontal circuits.

The immune response to stress
Effects of stress can lead to the inhibition of the immune system as a reaction to the HPA-mediated increased secretion of steroids. That phenomenon occurs due to compensatory activity of the HPA axis to stress. On the other hand, stress can also cause immune activation through other pathways. Hypothalamic corticotropin releasing factor (CRF) can independently stimulate noradrenaline release via CRF-receptor sites in the locus ceruleus, which activates the sympathetic nervous system, both within the central and peripheral nervous systems, and increase the release of adrenaline from the adrenal marrow. Thus, stress may cause immune activation including the release of humoral immune factors, such as interleukins (IL)-1 and IL-6 which further cause the release of CRF, which theoretically affects the increase of the glucocorticoid effect and thus limits immune activation.

There is evidence to support that patients exposed to chronic stress are at an increased risk for some physical diseases. The current literature data indicate that chronic stress may increase the risk of gastrointestinal diseases (irritable colon, ulcerative colitis, peptic ulcer), cardiovascular diseases (high blood pressure, arrhythmia, vasovagal syncope), respiratory diseases (hyperventilation syndrome, asthma, chronic obstructive pulmonary disease), endocrine diseases (hyper- and hypothyroidism, diabetes mellitus, hypercortisolism and hyperprolactinemia), dermatologic diseases (psoriasis, atopic dermatitis, psychogenic excoriation, pruritus), musculoskeletal diseases (rheumatoid arthritis and systemic lupus) and headaches (migraine, cluster and tension).

Psychosomatic medicine
Psychosomatic medicine is a specific field of medicine exploring the relationships among biological, psychological and social factors which need to be taken into account when considering the etiology and treatment of diseases [16, 17]. It is defined as a medical field dealing with illnesses in whose etiology and course psychological factors play critical or at least

important role. The importance of the interaction between the mind and body was observed in early history. Psychosomatic medicine has evolved from the time of Hippocrates, when the disease was considered together with religious aspects, when the role of emotions and adaptive capabilities in etiology and course of some somatic diseases was originally observed. In the 5th century before Christ (BC), Socrates concluded that the body cannot heal if the soul is left untreated, while Galen underscored the concept of adaptive biology in which disease disturbs the balance of body [18]. The word psychosomatic was first used by a German doctor Heinroth in 1818, when he wanted to highlight the role of psychological factors in somatic illness and vice versa [19, 20]. In the beginning of the 20th century, Walter Cannon, an American scientist, studied physical changes in different mental states, especially in situations of biological vulnerability and established one of the basic psychophysiological laws "fight or flight" [21]. While observing animals in danger, he noted the presence of piloerection, mydriasis, tachycardia, expanding air routes and concluded that these phenomena are intended to overcome the danger. In the first half of the 20th century, another American scientist, Helen Dunbar, studied a large number of patients with somatic diseases and noticed some similar personality traits in patients with similar somatic diseases. She concluded that people with specific personality characteristics are subject to the development of specific diseases and in that sense described coronary, ulcer and arthritic personality [22]. Franz Alexander, an American psychoanalyst, at the same time emphasized the importance of individual psychodynamic conflict in the etiology of psychosomatic diseases neglecting the personality characteristics of patients. In his opinion, a chronic irritation of the parasympathetic nervous system as a result of taking a passive stance and withdrawing from life's struggles over the years may lead to the development of asthma, colitis and fatigue [15]. On the other hand, vegetative neurosis, which uses a sympathetic pathway, may lead to migraine, diabetes mellitus and cardiovascular diseases [18]. Other psychoanalysts, George Angel and Arthur Schmale, concluded that inadequate mourning after the loss may be a precipitating factor for the development of psychosomatic diseases [23]. A pioneer of consultation psychiatry, American psychiatrist Lipowski, defined this field as a scientific consideration of the relationship between the physiological, biological and social factors in health and disease. In his opinion, a holistic approach to medicine occurs only through consultation liaison psychiatry, which is an integral part of psychosomatic medicine [24].

Clinical implications of psychosomatic medicine
In the interdisciplinary field of psychosomatic medicine, psychosocial factors, affecting individual's vulnerability to diseases, include life events and allostatic load, health attitudes and behaviors, social support, psychological well-being, spirituality and personality [25]. The biopsychosocial approach, which takes into account both biological and psychosocial aspects when considering the diagnosis, treatment and rehabilitation of patients, unfortunately has not yet been adopted in modern medicine. Psychological and psychosomatic medicine point to high incidence of mental disorders in general population, especially in patients with somatic diseases. In clinical sense, psychosomatic medicine deals with the diagnosis and treatment of patients with comorbid psychiatric and somatic illnesses, patients with somatoform and functional disorders, and patients with psychiatric disorders which are a direct result of the somatic disease or its treatment. Psychosomatic medicine has become a discipline based on empirical research and spread its results into many fields of medicine [26]. The importance of psychosomatic medicine is highlighted by the fact that this discipline is the latest psychiatric subspecialty, formally approved by the American Board of Medical Specialists.

Conclusion

This review has shown the significance of psychological and social factors in the prevention, etiology, treatment and course of many medical disorders. However, present barriers, in terms of separation of mental healthcare from somatic medicine, still remain a significant clinical problem in many health conditions where psychological factors are essential. Because of their importance to the well-being of patients, psychological and psychosomatic medicine should be an integral part of the clinicians' knowledge and professional skills across all medical specialties.

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Erratum

Hereby we offer our apologies for an inadvertent error published in the *Medical Review: 5-6/2019*, on page 171 and in the contents, where Sanja Jovanović was listed as a co-author instead of Sandra Jovanović.
Editorial Board

Izvinjavamo se zbog nenamerno učinjene greške u dvobroju 5-6/2019 na strani 171 i u sadržaju gde u navođenju koautora umesto Sandra Jovanović navedeno Sanja Jovanović.
Uredništvo

CASE REPORTS
PRIKAZI SLUČAJEVA

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SABER-SHEATH TRACHEA AS A CHALLENGE FOR SAFE SURGERY – A CASE REPORT

„SABER-SHEATH" TRAHEJA KAO IZAZOV ZA BEZBEDNU HIRURGIJU – PRIKAZ SLUČAJA

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Marija D. MARINKOVIĆ[1, 2] and Vladimir Đ. DOLINAJ[1, 3]

Summary

Introduction. Saber-sheath trachea is a rare malformation of the trachea marked with coronal narrowing with concomitant widening of sagittal diameter, with a sagittal-to-coronal diameter ratio exceeding 2:1. As tracheal rings are stiff and do not collapse around the tube, the intubation is difficult. **Case Report.** A 53-year-old female was referred to our hospital due to failed intubation at the local hospital during a planned surgery of skin melanoma. The patient was scheduled for surgery in our hospital, her case was presented to a panel of anesthesiologists and she was prepared for surgery. The intubation failed again, even though a video-assisted laryngoscope and endotracheal tube with 5 mm internal diameter was used. The anesthesiologist noticed that the endotracheal tube entered the trachea only 2 cm due to strong resistance, so further intubation was not an option in order to avoid damaging the trachea. The surgery was performed in local anesthesia with analgosedation. Later on, computed tomography was done, because of suspected pathological process compressing trachea, but the radiologist described the anomaly as saber-sheath trachea. One year later, the patient presented with axillary lymph node metastases and needed another surgery. This time the anesthesiologist knew about the trachea malformation, anticipated difficult intubation and used i-gel[TM] for airway management. **Conclusion.** Despite the fact that numerous methods have been developed for the purpose of identifying patients at risk of difficult intubation, there are many unexpected airway pathologies that can lead to failed intubation. Adequate preoperative assessment, knowledge of Guidelines for Difficult Intubation Management, availability of supraglottic airway devices, and cooperation between the surgeon and anesthesiologist, are crucial to successful patient management.
Key words: Trachea; Cartilage; Intubation, Intratracheal; Anesthesia, General; Airway Obstruction; Tracheal Stenosis; Perioperative Care; Airway Management; Laryngeal Masks

Sažetak

Uvod. *Saber-sheat* traheja je retka malformacija traheje za koju je karakteristično suženje koronalnog, a proširenje sagitalnog dijametra u odnosu 2 : 1. Trahealni prstenovi su zadebljali, te traheja nije kompresibilna, a intubacija je otežana. **Prikaz slučaja.** U našu bolnicu je upućena 53-godišnja žena zbog neuspešne intubacije u regionalnoj bolnici, gde je pripremana za operaciju melanoma kože. U našoj ustanovi ovaj slučaj je prezentovan konzilijumu anesteziologa i pacijentkinja je pripremljena za operaciju. Ni ovaj put anesteziolog nije uspeo da intubira pacijentkinju, iako je korišćen videolaringoskop i endoktrahealni tubus unutrašnjeg dijametra od 5 mm. Uočeno je da tubus ulazi svega 2 cm u traheju, nakon čega se nailazi na veliki otpor, a zbog čega se odustalo od dalje intubacije kako bi se izbegla povreda traheje. Operacija je urađena u lokalnoj anesteziji uz analgosedaciju. U postoperativnom periodu urađena je kompjuterizovana tomografija vrata i grudnog koša zbog sumnje da neki patološki proces u grudnom košu pritiska traheju, ali je radiolog opisao anomaliju označenu kao *saber-sheat* traheja. Godinu dana kasnije, kod bolesnice su se razvile metastaze u pazušnim limfnim čvorovima i ponovo je bila neophodna operacija. Ovaj put anesteziolog je bio upoznat sa malformacijom traheje, očekivao je otežanu intubaciju i uspešno obezbedio disajni put korišćenjem *i-gel[TM]* supraglotičnog sredstva. **Zaključak.** I pored raznih metoda koje su razvijene sa ciljem identifikovanja prediktora teške intubacije, postoje brojni neočekivani patološki procesi traheje koji mogu da onemoguće intubaciju. Adekvatna preoperativna procena, poznavanje preporuka za problematičan disajni put, dostupnost supraglotičnih sredstava i saradnja hirurga i anesteziologa značajni su koraci ka uspešnom tretmanu pacijenta.
Ključne reći: traheja; hrskavica; intratrahealna intubacija; opšta anestezija; opstrukcija disajnih puteva; stenoza traheje; perioperativna nega; zbrinjavanje disajnih puteva; laringealna maska

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Introduction

Establishment of safe airway and good ventilation during operation is essential for every surgery. Numerous guidelines and morphometric tests have been developed in order to preoperatively identify patients who are at risk of difficult intubation, but half of difficult intubations are still unexpected [1–3]. Any kind of tracheal malformation, congenital or acquired, that is unrecognized before surgery may cause intubation failure and postpone the surgical procedure. On the other hand, forced intubation through a narrowed trachea can lead to tracheal rupture, uncommon, but a very serious complication [4, 5]. The shape of trachea is variable and can have different configurations at multiple levels [6].

One of the abnormal morphological shapes is saber-sheath trachea [7]. The saber-sheath trachea is defined as intrathoracic coronal narrowing with concomitant widening of the sagittal diameter, with a sagittal-to-coronal diameter ratio exceeding 2:1. At the same time, the extrathoracic trachea is normal. A deformed trachea is unlikely to collapse and it makes endotracheal intubation difficult. This malformation is more common in males and is usually associated with chronic obstructive pulmonary disease (COPD) [8].

We report a female patient undergoing surgery for melanoma that was twice preoperatively evaluated by anesthesiologists and was not identified as potentially difficult intubation, but later on, after two unsuccessful intubation attempts, she was diagnosed with saber-sheath trachea.

Case Report

A 53-year-old female was referred to the Clinical Center of Vojvodina due to unsuccessful attempt of intubation in a local hospital. She needed a surgery for a rapidly growing skin tumor that was diagnosed as melanoma. The patient underwent a standard preparation for this type of surgery. The preoperative chest X-ray was unremarkable. The patient was a non-smoker, with controlled hypertension, body mass index $34,34 \text{ kg/m}^2$ (body weight – 89 kg, height - 161 cm, obese class I), American Society of Anesthesiologists (ASA) III, Mallampati class II, Patil > 6 cm, neck circumference 39 cm, no evidence of obstructive sleep apnea or snoring. Preoperative assessment of this patient did not indicate potential intubation problems. The patient was scheduled for radical surgery in general anesthesia, but the surgery was canceled due to unsuccessful intubation. No further diagnostic procedures were done, and the patient was referred to a tertiary health center. Two weeks later, the patient was scheduled for surgery and was admitted to the Clinic

of Plastic and Reconstructive Surgery. Her case was presented to a panel of anesthesiologists, and besides standard preparation, an opinion of an ear-nose-throat specialist was requested. Indirect laryngoscopy was unremarkable. The intubation failed again, even though a video-assisted laryngoscope and endotracheal tube (ETT) with 7 mm internal diameter (ID) was used, then 6 mm ID, and finally 5 mm ID. According to Cormack-Lehane classification, the patient was grade I. The anesthesiologist noticed that ETT was entering trachea only 2 cm and then there was a strong resistance so she did not want to force further intubation in order to avoid tracheal injury. The patient was operated under local anesthesia with analgosedation. Te anesthesiologist indicated that further diagnostic procedures should be done in order to evaluate the tracheal lumen and a potential pathologic process in the thorax. A computed tomography (CT) was done and the radiologist described the anomaly called saber-sheath trachea **(Figure 1)**. There was a coronal narrowing of the intrathoracic portion of trachea with widening of the sagittal diameter at some levels
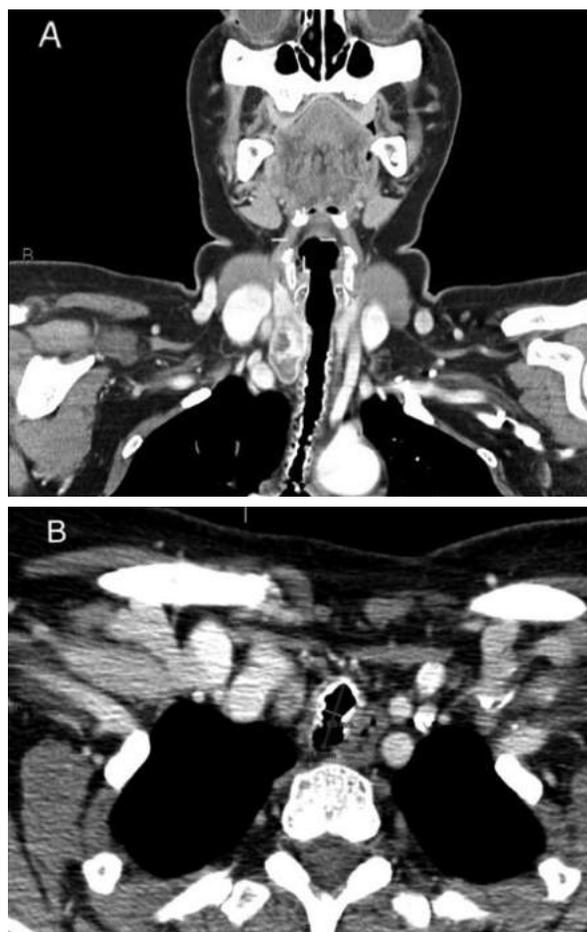

Figure 1. Saber-sheath deformity of the trachea; computed tomography scans: A) axial view, B) coronal view
Slika 1. „Saber-sheath” deformitet traheje – kompjuterizovana tomografija: A) aksijalni presek, B) koronalni presek

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Figure 2. Computed tomography scan of saber-sheath tracheal diverticula (axial view, arrows)
Slika 2. Kompjuterizovana tomografija divertikula „saber-sheath" traheje (aksijalni presek, strelice)

reaching 3:1 (18 x 6 mm). The findings also showed ossification of tracheal rings with thickening of the tracheal wall (maximum 6 mm) and irregular inside and outside tracheal contours with a few diverticula **(Figure 2)**.

One year later, the patient presented with axillary lymph node metastases and had to undergo surgery again. This time the surgeon and anesthesiologist knew about the tracheal malformation, anticipated difficult intubation and planned a different approach to anesthesia.

In the operating room, after preoxygenation of the patient in supine position, she was given premedication, 1 mg of midazolam and 50 μg of fentanyl. Anesthesia was induced with 200 mg of propofol. The next step was introducing a supraglottic airway device i-gel™ No 4, that was done easily and fixed to the mouth by using adhesive tapes. A muscle relaxant was not used, as difficult airway management was expected. The maintenance of anesthesia was done using sevoflurane MAC 2, 100 μg of fentanyl and oxygen-air mixture 50:50. Anesthesia apparatus settings were tidal volume 8 ml/kg and 12 breaths per minute. The patient was ventilated mechanically without noticeable air leakage during the surgery. After finishing the surgery, i-gel™ was removed and the patient regained consciousness without any respiratory problems afterwards.

Discussion

Safe airway management is a predisposition for every surgery requiring endotracheal intubation. Any kind of malformation of the trachea can be challenging for an anesthesiologist, especially if unrecognized before entering the operating room [9]. Standard preoperative imaging, if not required differently, generally includes only a chest X-ray, but tracheal malformations may not always be apparent, as in this case.

Difficult intubation with apparent airway obstruction, as in our patient, raises suspicion of some benign or malignant intrathoracic process or some disease that can affect the trachea, especially bearing in mind that this patient was already diagnosed with a malignant tumor - melanoma [10]. Even though the patient presented with a malignant disease, she had no significant respiratory problems, so there was no reason for performing a CT instead of chest X-ray routinely, as part of preoperative preparation. Later on, the CT showed a saber-sheath trachea. As can be seen from the literature, CT is the imaging modality of choice for trachea [11–14]. It shows the tracheal anatomy, but also enables good evaluation of adjacent mediastinal structures and intrathoracic masses that may compress the trachea or invade its walls. It also provides important morphological information concerning vascular elements and calcification of structures that can be important in recognition of some particular pathology.

This malformation is commonly associated with COPD, emphysema, chronic bronchitis and bronchiolitis obliterans syndrome after lung transplantation [15, 16]. It is believed that in COPD patients recurrent coughing and elevated intrathoracic pressure cause tracheal injures repeatedly. Degeneration and calcification of the cartilaginous rings during the healing process lead to remodeling and bending of rings that are seen in this specific deformity. The trachea becomes rigid and inflexible.

Our patient was a non-smoker and did not have COPD or any other kind of respiratory disease in her medical history. Her chest X-ray was described as regular, so there was no reason for additional imaging procedures during a standard preparation for melanoma surgery. The problem with intubation occurred twice, in two different hospitals, with two different doctors trying to perform intubation and both made the same remark on tracheal obstruction. We assumed that there was some intrathoracic process, some constricting mediastinal mass, possible secondary deposits of melanoma or something else that had to be further diagnosed.

Tracheal stenosis influences the clinical decision making process with regard to the mode of intubation, whether to use some supraglottic airway device or ETT, which size of ETT to use, and if there is a need for video- or fibre-optically guided intubation. From the anesthesiologists' point of view, there are many problems connected with this deformity of the trachea. The most important issue is placement of ETT through a narrow and stiff trachea. If this problem is successfully solved, there is a possibility of air leaking as circular shaped balloon on endotracheal tube cannot obliterate perfectly the tracheal cavity because of the sagittal elongation of trachea. In order to avoid forcing the ETT through the obstructed trachea, a supraglottic airway device should be used whenever possible [17, 18]. The decision making process in difficult airway management can be challenging and depends on multiple factors, such

as urgency of the operation, comorbidities, doctor's skills, availability of supraglottic airway devices, team coordination. The failure of ETT placement is not a personal deficiency. It is just a decision to "stop and think" and take the next step. Limiting the number of airway intervention attempts is important, because every attempt may potentially cause a trauma. We cannot overemphasize the importance of guidelines that clearly lead us from step to step in order to avoid our stress induced cognitive overload to result in a bad decision [3].

Conclusion

It is important to recognize saber-sheath trachea and explain to the patient the potential problems in the future. Also, all the problems that occur during surgery and general anesthesia need to be clearly marked in medical records, whereas the patient must be instructed about them in order to make proper diagnosis in the future. In this way, any potential problem in subsequent operations may be anticipated and the anesthesiologist can decide on the best option for secure airway management during general anesthesia without unnecessary stress and unpleasant surprises in the operating room. Failed intubation can lead to great morbidity during anesthesia and it is potentially life-threatening. Adequate preoperative assessment, knowledge of guidelines for difficult intubation, availability of second generation devices, such as video laryngoscope, supraglottic airway devices, calm head and cooperation between the surgeon and anesthesiologist, are important steps to safe patient management.

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Knjige i druge monografije
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6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

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8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals
* *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.
* *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.
* *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.
* *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.
* *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.
* *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs
* *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.
* *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.
* *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.
* *A conference paper*

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

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material
* *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle
* *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.
* *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).
THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements
SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.