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Vojnosanitetski Pregled



# VOJNOSANITETSKI PREGLED

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## CONTENTS / SADRŽAJ

### EDITORIAL / UVODNIK

*Rajko Igić*

#### **Smoking and COVID-19**

Pušenje i COVID-19..... 461

### ORIGINAL ARTICLES / ORIGINALNI RADOVI

*Darija Kisić Tepavčević, Milena Kanazir, Gorica Marić, Milica Zarić, Goranka Lončarević, Tatjana Gazibara, Nataša Maksimović, Tatjana Pekmezović*

#### **Hepatitis B-related awareness among health care workers in Belgrade, Serbia**

Znanje zdravstvenih radnika o hepatitisu B u Beogradu, Srbija ..... 463

*Milica Popović, Suzana Živanović, Tamara Vučićević, Miona Grujović, Miloš Papić*

#### **Cone-beam computed tomography study of tooth root and canal morphology of permanent molars in a Serbian population**

Analiza morfologije korenova i kanala korenova stalnih molara primenom kompjuterizovane tomografije konusnog zraka u populaciji Srbije ..... 470

*Petar Dabić, Saša Borović, Predrag Milojević, Jelena Kostić, Zoran Trifunović, Rade Babić*

#### **Evolution of concomitant moderate and moderate to severe functional mitral regurgitation following aortic valve surgery for severe aortic stenosis**

Evolucija istovremene umerene i umerene do teške funkcionalne mitralne regurgitacije nakon operacije teške stenozе aortnog zaliska ..... 479

*Marina Kovačević-Lepojević, Zoran Ilić, Marija Maljković, Milica Kovačević, Ljeposava Ilijić*

#### **Parenting and externalising problems in adolescents: results of factor analysis**

Roditeljstvo i eksternalizovani problemi adolescenata: rezultati faktorske analize ..... 487

*Ena Grbović, Boban Mugoša*

#### **Social support in recovery former users of psychoactive substances in Montenegro: A survey study**

Socijalna podrška u periodu oporavka bivšim korisnicima psihoaktivnih supstanci u Crnoj Gori: Istraživačka studija ..... 494

*Zoran Šegrt, Slavica Vučinić, Nemanja Rančić, Nataša Vukčević-Perković, Gordana Vuković-Ercegović, Olivera Potrebić, Aleksandra Kovačević, Vesna Mijatović Jovin, Vesna Jačević*

#### **Principal characteristics of patients acutely poisoned by ethanol in the region of Belgrade (Serbia)**

Osnovne karakteristike pacijenata akutno otrovanih etanolom na području Beograda (Srbija)..... 500

*Nedim Hamzagić, Marija Andjelković, Marijana Stanojević Pirković, Petar Čanović, Vesna Ignjatović, Amra Ramović, Dejan Petrović*

#### **Influence of secondary hyperparathyroidism in management of anemia in patients on regular hemodialysis**

Uticaj sekundarnog hiperparatireodizma na lečenje anemije kod bolesnika na hroničnom programu hemodijalize ..... 508

*Vesna Djordjević, Marija Denčić-Fekete, Jelica Jovanović, Ivan Soldatović, Gradimir Janković, Andrija Bogdanović*  
**Significance of cytogenetic-risk categories and refined international prognostic scoring system for overall survival in primary myelofibrosis: A single-center experience**

Značaj citogenetički rizičnih kategorija i prerađenog Međunarodnog prognostnog sistema za procenu ukupnog preživljavanja u primarnoj mijelofibrozi: iskustvo jednog centra..... 516

*Djordje Taušan, Zoran Kostić, Damjan Slavković, Branimir Nešković, Dubravko Bokonjić, Sandra Šipetić-Grujičić, Nenad Ratković, Vesna Šuljagić*

**Incidence, in-hospital mortality and risk factors for hospital-acquired pneumonia in patients with intra-abdominal surgical procedures hospitalized in a tertiary hospital in Belgrade, Serbia: A matched case-control study**

Incidenca, bolnička smrtnost i faktori rizika od nastanka bolnički stečene pneumonije kod bolesnika nakon intraabdominalnih hirurških procedura, hospitalizovanih u bolnici tercijernog nivoa zdravstvene zaštite u Beogradu, Srbija: *case-control* studija ..... 525

*Igor Krdžić, Marko Kenić, Milena Šćepanović, Ivan Soldatović, Jelena Ilić Živojinović, Dragan Radovanović*

**Parameters of radical resection in laparoscopic and open colon and rectal cancer surgery**

Parametri radikalnosti kod laparoskopske i otvorene hirurgije karcinoma kolona i rektuma ..... 532

*Galina Stevanović, Nebojša Jović, Miljana Kecmanović*

**Is adjunctive perampanel beneficial for Lafora disease?**

Da li je primena perampanela korisna u lečenju Laforine bolesti? ..... 539

CASE REPORTS / KAZUISTIKA

*Vladimir Harhaji, Ivica Lalić, Miodrag Vranješ, Milena Mikić, Vladimir Djan*

**Isolated avulsion fracture of the fibular head**

Izolovani avulzioni prelom glave lisnjače ..... 545

*Rade Babić, Goran Grujić, Dejan Kojić, Jelena Kostić, Zoran Trifunović, Saša Borović*

**Primary percutaneous intervention of the right coronary artery in a setting of anomalous origination of left coronary artery from the opposite sinus of Valsalva**

Primarna perkutana intervencija desne koronarne arterije u okolnostima anomalnog porekla leve koronarne arterije iz suprotnog sinusa Valsalva ..... 549

*Tatjana Ivković Kapić, Ferenc Vicko, Lazar Popović, Dragana Djilas, Tanja Lakić*

**Secretory breast carcinoma in adulthood – A case report with literature review**

Sekretorni karcinom dojke u odraslom dobu ..... 556

IN MEMORIAM

Branislav–Backo Popović (1931–2020) ..... 560

INSTRUCTIONS TO THE AUTHORS / UPUTSTVO AUTORIMA..... 562



Every year, on 31 May, the World Health Organization (WHO) celebrates World No Tobacco Day. It is an opportunity to raise awareness on the harmful and deadly effects of tobacco use and second-hand smoke exposure, and to discourage the use of tobacco in any form.

This year, World No Tobacco Day falls during the coronavirus pandemic. There are still no reliable data on the influence of smoking on the risk of COVID-19 and the severity of its clinical picture. However, as smoking impairs lung function, and COVID-19 is primarily a respiratory tract infection, smoking may be an additional aggravating factor for this disease (see Editorial, pp. 461–462).

Svake godine, 31. maja, Svetska zdravstvena organizacija obeležava Svetski dan bez duvanskog dima. To je prilika za podizanje svesti o štetnim i letalnim efektima direktnog i pasivnog izlaganja duvanskom dimu sa ciljem obeshrabrivanja upotrebe duvana u bilo kom obliku.

Ove godine Svetski dan bez duvanskog dima pada u vreme pandemije koronavirusa. Još uvek nema pouzdanih podataka o uticaju pušenja na COVID-19 i težinu kliničke slike ove infekcije. Međutim, kako pušenje oštećuje funkciju pluća, a COVID-19 je prvenstveno infekcija respiratornog trakta, pušenje može da bude dodatni otežavajući faktor za tu bolest (vidi Uvodnik, str. 461–462).



## Smoking and COVID-19

## Pušenje i COVID-19

Rajko Igić

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The coronavirus (SARS-CoV-2) pandemic is still progressing in most countries, and another wave of infection could be expected this fall. However, we do not know enough about various factors that affect COVID-19, a disease caused by this virus. For example, it is not known if the virus affects smokers and non-smokers in the same way or to the same extent.

It is well known that smoking damages the immune system and lung tissues. As a result, chronic smokers are more sensitive to infectious agents. They are twice as likely to get the flu, and flu symptoms are usually more severe in smokers. Tobacco smoking is also clearly associated with lung cancer and chronic obstructive pulmonary disease. In Serbia, the incidence of smoking is slowly decreasing. Two useful health actions possibly blunted this harmful habit. A long time ago, one was introduced in Yugoslavia by medical students from Tuzla and Banja Luka (January 31, the Non-Smoking Day) several years before the World Health Organization determined that May 31 would be the World NoTobacco Day<sup>1,2</sup>.

In a previous coronavirus (MERS-CoV) outbreak in 2012, smokers had higher mortality than nonsmokers<sup>3</sup>. It is thus important to establish the effect of tobacco smoking on the outcomes of infection with COVID-19. Since the beginning of the current pandemic, Chinese doctors sought a relationship between smoking and this viral disease. Accordingly, they measured not only mortality but also the number of infections in both smokers and non-smokers, the ratios of the number of these patients who required intensive care to those who did not, and how many required mechanical respiration.

In one of five such studies conducted in Wuhan<sup>4</sup>, 191 patients were followed, 137 survived and 54 died. Among the dead, 9% were smokers and 4% were non-smokers. Another, more extensive study<sup>5</sup> showed that smokers were 1.4 times more likely to have severe symptoms requiring

placement in intensive care units with mechanical respiration. Although these, and most other published results, indicate a negative impact of smoking on this viral disease<sup>6</sup>, one exception is the study by Lippi and Henry<sup>7</sup> of Chinese patients that was based on preliminary meta-analysis. They found that smoking did not seem to be significantly associated with enhanced risk of severe COVID-19 disease. Further research should determine whether the impact is significant in better-designed studies.

Because there are many smokers in some parts of Serbia, a large number of people are at risk for this viral disease. It would be worthwhile to establish the severity of COVID-19 disease in several populations: smokers, passive smokers (people who live with a smoker in the household or work in smoking rooms), former smokers (those who quit smoking more than a year ago) and non-smokers. This will determine how our population reacts to exposure to tobacco smoke.

In addition to the effects of tobacco smoke on the spread of coronavirus and the severity of COVID-19 infection, it is worth noting that smoking stimulates the liver enzymes, cytochrome P450 (CYP) 1A2 and CYP2B6, that metabolize various drugs, including chloroquine and hydrochloroquine. Hospitalized patients rapidly become nonsmokers because smoking is prohibited there. In addition to abstinence problems, the activities of liver enzymes gradually decrease, and in a week enzyme activity approximates that of non-smokers<sup>8</sup>. If a smoker takes a drug that is metabolized in the liver before coming to the hospital, and continues with the same higher dose, after becoming an enforced non-smoker, it may cause toxic effects. These antimalaria drugs have not even been proven effective for resolution of COVID-19.

In this time of home isolation and social distancing, many people tend to increase their cigarette consumption,

including the relapse of former smokers<sup>9</sup>. Pandemic management teams, including all doctors, should openly address this problem so that the general public becomes aware of the risks. In addition to information provided about the harmfulness of increased cigarette consumption for the outcome of COVID-19 infection, smokers should be advised

to reduce or quit using cigarettes. Ex-smokers might rely on non-pharmacological procedures, or they might temporarily use pure nicotine pharmaceutical preparations or the nicotine antagonist, bupropion. Bupropion reduces nicotine dependence and may be used in an abstinence crisis<sup>10</sup>.

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# Hepatitis B-related awareness among health care workers in Belgrade, Serbia

## Znanje zdravstvenih radnika o hepatitisu B u Beogradu, Srbija

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### Abstract

**Background/Aim.** Despite the availability of safe and effective vaccine since 1982, hepatitis B virus (HBV) infection still remains a major occupational disease among health care workers (HCWs) worldwide. The aim of this study was to estimate the level of knowledge regarding HBV among HCWs in Serbia. **Methods.** A random sample of 352 HCWs, stratified by occupation, was selected from the list of employees in the Clinical Centre of Serbia during December 2015. Anonymous questionnaire was used in data collection. Data were statistically assessed. **Results.** The mean HBV knowledge score was  $22.9 \pm 4.8$  (out of maximum 30). Better knowledge score correlated with higher education attainment ( $\rho = 0.377$ ;  $p < 0.001$ ), younger age ( $\rho = -0.113$ ;  $p = 0.034$ ) and less working experience ( $\rho = -0.127$ ;  $p = 0.017$ ). We observed that those HCWs who were previously vaccinated against hepatitis B demonstrated statistically significantly higher knowledge level ( $t = 5.656$ ,  $p < 0.001$ ) compared to HCWs who were not vaccinated ( $23.9 \pm 3.7$  vs.  $20.9 \pm 5.8$ , respectively). **Conclusion.** We found poor level of knowledge on some aspects of HBV infection among HCWs in Serbia. Tailoring of education campaigns in this high-risk exposure group is necessary. Education intervention should focus on change of attitudes and behaviour modification to ensure safe and responsible health care environment.

### Key words:

hepatitis b; medical staff; education; infection control; serbia.

### Apstrakt

**Uvod/Cilj.** Uprkos dostupnosti sigurne i efektivne vakcine još od 1982. godine, infekcija virusom hepatitisa B (HBV) i dalje predstavlja glavno profesionalno oboljenje među zdravstvenim radnicima širom sveta. Cilj ovog istraživanja je bio procena znanja o HBV-u u populaciji zdravstvenih radnika u Srbiji. **Metode.** Slučajni uzorak od 352 zdravstvena radnika, stratifikovan u odnosu na profesiju, je odabran sa spiska zaposlenih Kliničkog Centra Srbije tokom decembra 2015. godine. Za prikupljanje podataka je korišćen anonimni upitnik. Podaci su statistički obrađeni. **Rezultati.** Prosečan skor znanja o HBV-u je iznosio  $22,9 \pm 4,8$  (od maksimalnih 30). Viši nivo znanja je korelirao sa višom stručnom spemom ( $\rho = 0,377$ ;  $p < 0,001$ ), mlađim uzrastom ( $\rho = -0,113$ ;  $p = 0,034$ ) i kraćim radnim stažom ( $\rho = -0,127$ ;  $p = 0,017$ ). Zapaženo je da su zdravstveni radnici koji su prethodno vakcinisani protiv hepatitisa B imali statistički značajno viši nivo znanja ( $t = 5,656$ ,  $p < 0,001$ ) od onih koji nisu bili vakcinisani ( $23,9 \pm 3,7$  naspram  $20,9 \pm 5,8$ ). **Zaključak.** Zapažen je nizak nivo znanja zdravstvenih radnika u Srbiji u vezi sa pojedinim aspektima HBV-a. Neophodno je kreiranje obrazovnih kampanja za ovu populacionu grupu izloženu visokom riziku od infekcije. Obrazovna intervencija bi trebalo da ima za cilj promenu stavova i ponašanja kako bi se obezbedila sigurna i odgovorna zdravstvena zaštita.

### Ključne reči:

hepatitis b; kadar, medicinski; obrazovanje; infekcija, kontrola; srbija.



## Introduction

Although a safe and effective hepatitis B virus (HBV) vaccine has been available for the past 4 decades, hepatitis B infection remains a major occupational disease among health care workers (HCWs) globally<sup>1-4</sup>. A number of studies have previously reported that acceptance of vaccine against HBV among HCWs varies. Specifically, the lowest coverage was reported in African region (approximately 15%), while coverage of more than 75% has been reported in Australia, New Zealand and the USA<sup>5-9</sup>. These remarkable global variations most likely originate from discrepancy in awareness of exposure risk. Indeed, perception of HBV infection risk has been strongly associated with general preventive behaviours in this susceptible cohort<sup>5,10</sup>.

Regardless of well-recognized professional risk for HBV infection, it has been emphasized that poor level of preventive practices among HCWs appear difficult to determine and explain<sup>10,11</sup>. Furthermore, studies conducted in both developed<sup>12,13</sup> and developing<sup>14</sup> countries documented insufficient HBV knowledge among HCWs. Overall, lower HBV-related awareness has been associated with poorer preventive attitudes, including decrease in HBV vaccination coverage<sup>15</sup>. Lack of awareness regarding risk of exposure to HBV has been particularly prominent among HCWs in developing countries. Subsequently, lack of awareness promotes low HBV vaccination coverage. Moreover, estimates suggest that in developing countries 40–60% of HBV infection in HCWs has been attributed to professional hazard. On the other hand, in developed countries, the attributed fraction of HBV infection is less than 10%, as a result of higher vaccination coverage<sup>1</sup>.

In the Republic of Serbia determinants of hepatitis B-related awareness in health care setting have not been sufficiently explored and understood. Therefore, we aimed at estimating the level of knowledge relative to HBV nature, transmission, complications and prevention among HCWs in a national health care centre in Serbia.

## Methods

### *Participants and settings*

This cross-sectional study was conducted at the Clinical Centre of Serbia in the capital city of Belgrade. The Centre is the largest hospital complex in the Republic of Serbia. It consists of 41 units of which 23 are clinics. There are a total of 3,500 beds available for in-patients. The Centre has been affiliated with the Faculty of Medicine, University of Belgrade, a state university with approximately 1,200 employees.

Since 1989 the HBV vaccine has been provided free of charge to the occupationally exposed workers at the Clinical Centre of Serbia. Also, the vaccine has been periodically provided (based on current socioeconomic circumstances and vaccine stocks) at first employment visit and at request. However, the HBV vaccine is compulsory after a high-risk occupational injury.

We selected a random sample of HCWs from the list of employees who were subsequently stratified by occupation.

The selection of study participants was conducted during December 2015. The structure of the study sample was based on the proportion of occupational distribution in the Centre (physicians/nurses/technicians/ administrative staff, etc). The sample comprised 7.1% of the employees at the Clinical Centre of Serbia.

All participants provided signed informed consents. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade.

### *Instruments*

Relevant data in this study were collected by a questionnaire, derived and adapted from others surveys<sup>11,16</sup>. After translation to Serbian language, its validity was assessed and previously published<sup>17</sup>. First set of questions explored demographic and occupational data (such as gender, age, marital status, type of occupation, work place and duration of work experience). Second set of questions comprised 30 statements (with binary yes/no options), assessing HCWs' knowledge on HBV infection. These items referred to the nature of HBV infection and its transmission, symptoms, complications, possibility for prevention and treatment. Each correct answer in this set of items was awarded 1 point. Thus, a possible range of knowledge score was from 0 to 30 points. To have a more detailed insight in knowledge level, the knowledge score was categorized based on median value (24 points). This means that median value was labelled as "good knowledge level". Therefore, observed values lower than median were labelled as "unsatisfactory", while values greater than median were additionally divided into two subcategories as follows: "very good knowledge level" (25–27 points) and "excellent knowledge level" (28–30). Third set of questions assessed hepatitis B vaccination status of the study participants, as well as the number of issues concerning potential contact with blood and blood products in the workplace.

### *Statistical analyses*

All continuous variables were given as mean  $\pm$  standard deviation. All categorical variables were presented as count and corresponding percentage. Differences between vaccinated and non-vaccinated groups of HCWs were assessed by *t*-test. The relationship between the HBV-related knowledge score and selected variables was assessed by Spearman's rank correlation coefficient. Probability level of  $p < 0.05$  was considered statistically significant.

## Results

We invited a total of 380 HCWs to participate in the study, and 367 (96.6%) agreed to participate. Nevertheless, 356 (93.7%) provided all relevant information necessary for the study. Among them, four potential participants reported a history of hepatitis B and, thus, were excluded from all subsequent analyses. Accordingly, the total study sample comprised 352 HCWs.

Basic socio-demographic and occupational characteristics of the study sample are presented in Table 1. Mean age of respondents was  $39.1 \pm 9.2$  years. There were 86 males and 266 females with sex ratio (males:females) of about 1:3. More than one half of participants (59.9%) were married.

Predominant occupation in our sample were nurses (51.4%) and physicians (26.1%), while most respondents worked in in-patient wards (31.8%) and operating rooms (29.8%). Mean work experience among study participants was  $15.9 \pm 10.1$  years.

**Table 1**  
**Socio-demographic characteristics of the study sample (n = 352)**

Variable	Value
Gender, n (%)	
male	86 (24.4)
female	266 (75.6)
Age (years)	
mean $\pm$ SD	$39.1 \pm 9.2$
range	21–62
Marital status, n (%)	
single (never married)	111 (31.5)
married/cohabiting	211 (59.9)
separated/divorced	28 (8.0)
widowed	2 (0.6)
Occupation, n (%)	
physicians specialists	46 (13.1)
physicians undergoing specialization	39 (11.0)
physicians without specialization	7 (2.0)
nurses	181 (51.4)
medical technologists	6 (1.7)
laboratory technologists	34 (9.7)
administrative staff	7 (2.0)
sanitary workers	14 (4.0)
others	18 (5.1)
Work site, n (%)	
operating room	105 (29.8)
emergency room, hemodialysis	37 (10.5)
specialty ward/intensive care unit	35 (9.9)
laboratory	6 (1.7)
inpatient wards	112 (31.8)
others	57 (16.2)
Duration of work experience (years)	
mean $\pm$ SD	$15.9 \pm 10.1$
range	0–36

**SD – standard deviation.**

Overall, 58.5% of HCWs in our study reported sharps injury, and 73.5% reported unprotected blood muco-cutaneous exposure in the past year. Furthermore, overall hepatitis B vaccination coverage was 66.2%. Potential reasons for HBV vaccine acceptance or refusal in this HCWs sample has been previously analyzed<sup>17</sup>.

The item-specific proportions of incorrect answers related to hepatitis B knowledge are displayed in Table 2. Approximately, one in three HCWs in our sample incorrectly identified recommendations on special diet for patients with hepatitis B, possibility for family transmission of HBV and potential for reinfection. Specifically, 125 HCWs (35.5%) inaccurately answered that newborns should not receive HBV vaccine. Additionally, more than one half of participants incorrectly considered that pregnant women should not receive HBV vaccine, that people with hepatitis B should

be restricted from working in the food industry, and that hepatitis B cannot lead to cirrhosis (Table 2).

Mean hepatitis B-related knowledge score was  $22.9 \pm 4.8$  out of 30 points. Distribution of HCWs according to hepatitis B knowledge level was presented in Figure 1. Almost one half of respondents (n = 175) scored less than median, suggesting “unsatisfactory” level of knowledge. On the other hand, 32 (9.1%) responders achieved “excellent” knowledge score. Small proportion of HCWs (5.4%) answered correctly to all questions (knowledge score 30 out of 30). By contrast, 2 (0.6%) participants provided only one correct answer. Higher level of hepatitis B-related knowledge correlated with higher education attainment ( $\rho = 0.377$ ;  $p < 0.001$ ), being younger ( $\rho = -0.113$ ;  $p = 0.034$ ) and having less work experience ( $\rho = -0.127$ ;  $p = 0.017$ ).

Table 2

## Proportions of incorrect hepatitis B-related knowledge answers

Statements	Incorrect answers, n (%)
<b>Transmission</b>	
HBV can spread from one person to another in the family.	162 (46.0)
Hepatitis B can be spread by sharing dishes with HBV positive patients.	96 (27.3)
HBV can be transferred through colonoscopy or endoscope tools.	94 (26.7)
Hepatitis B can be spread by mosquitoes.	78 (22.2)
Hepatitis B is spread through the air in an enclosed environment.	61 (17.3)
Hepatitis B can be transferred from mother to fetus.	45 (12.8)
Hepatitis B can be spread through close personal contact such as talking and kissing.	45 (12.8)
HBV can be transferred through mother's milk to the infant.	41 (11.6)
Having a medical and/or dental procedure increases a person's chances of contracting hepatitis B	33 (9.4)
Some people with hepatitis B were infected through unsterile tattooing.	30 (8.5)
Some people with hepatitis B were infected through blood transfusions.	24 (6.8)
Hepatitis B is commonly spread by sexual transmission.	20 (5.7)
Hepatitis B is spread through blood-to-blood contact.	16 (4.5)
Hepatitis B can be spread through sharing injecting equipment, such as needles and operation tools.	15 (4.3)
<b>Nature and complications</b>	
Hepatitis B is caused by a virus.	18 (5.1)
Hepatitis B can lead to cirrhosis.	208 (59.1)
Hepatitis B is associated with an increased risk of liver cancer.	101 (28.7)
Symptoms of hepatitis B infection always appear.	89 (25.3)
A person can be infected with HBV and not have any symptoms of the disease.	84 (23.9)
After the entry of HBV to the body, symptoms appear after 1 to 3 days.	51 (14.5)
An individual can have hepatitis B antibodies without being currently infected with the virus.	44 (12.5)
Once you have had hepatitis B, you cannot catch it again because you are immune.	143 (40.6)
<b>Prevention</b>	
Pregnant women should not receive the vaccine against hepatitis B.	181 (51.4)
People with hepatitis B should be restricted from working in the food industry.	179 (50.9)
Newborn children should not receive the vaccine against hepatitis B.	125 (35.5)
Special diet is recommended for patients with hepatitis B.	120 (34.1)
Vaccination against hepatitis B is obligatory for all persons employed in health care institutions that come in direct contact with infectious materials.	41 (11.6)
There is a vaccine for hepatitis B.	11 (3.1)
<b>Treatment</b>	
The vaccine can be used for the treatment of hepatitis B.	102 (29.0)
There is a pharmaceutical treatment available for hepatitis B.	61 (17.3)

## HBV – hepatitis B virus.

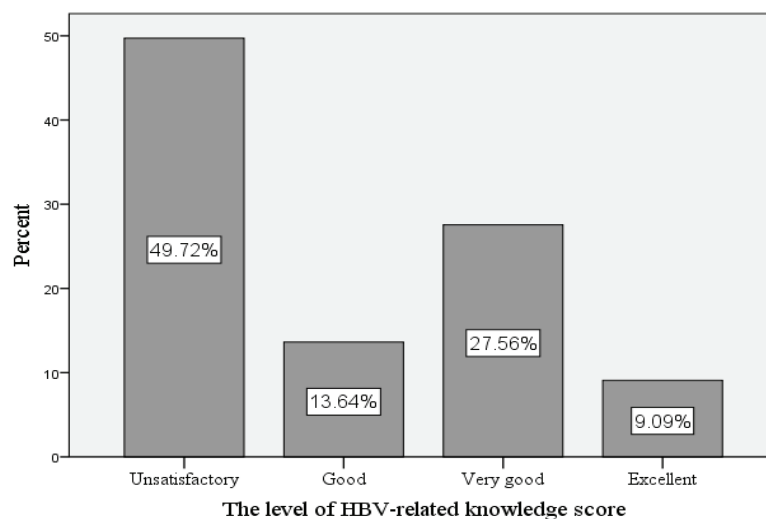


Fig. 1 – Distribution of hepatitis B-related knowledge level.

We observed that those HCWs who were previously vaccinated against hepatitis B, had significantly higher knowledge score ( $t = 5.656, p < 0.001$ ) compared to HCWs who were not vaccinated ( $23.9 \pm 3.7$  vs.  $20.9 \pm 5.8$ , respectively) (Figure 2). Additionally, the average value of

knowledge score was higher ( $t = 4.721, p < 0.001$ ) in subcohort of participants who reported occupational exposures to blood and body fluids ( $23.3 \pm 4.0$ ), compared to subcohort of HCWs who did not report this professional risk ( $19.3 \pm 5.4$ ) (Figure 3).

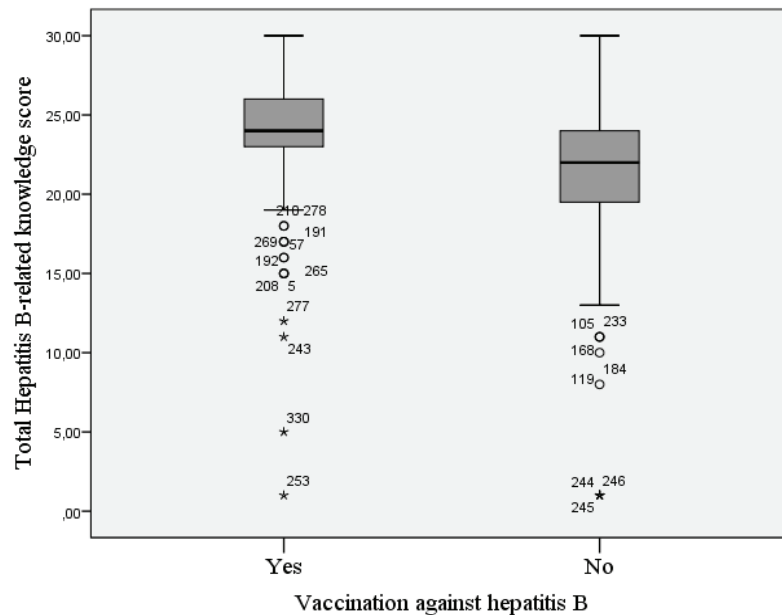


Fig. 2 – Total hepatitis B-related knowledge score according to the hepatitis B vaccination status.

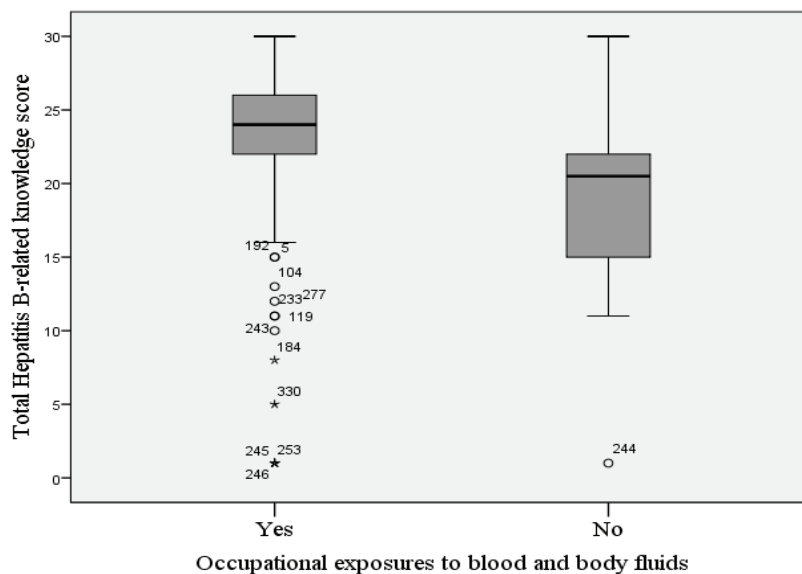


Fig. 3 – Total hepatitis B-related knowledge score according to the occupational exposures to blood and body fluids.

## Discussion

Regardless of well-recognized high professional risk for HBV infection and ample possibilities for effective prevention, results from evidence-based studies suggest that a considerable knowledge gap about hepatitis B still exists among HCWs. It has been indicated that insufficient HBV awareness in this professional group could profoundly influence their behavioural patterns towards vaccination and other

safety measures. Thus, testing the level of HCWs' hepatitis B awareness is of tremendous importance, considering the fact that knowledge is crucial factor for prevention-related behaviour modification. Keeping this public health problem in mind, over the past years increasing numbers of studies has been dealing with this particular issue<sup>9, 13, 14, 16, 18-20</sup>. Therefore, the present study conducted in Serbian health care setting aimed at addressing this challenge.

Slightly more than half of HCWs in our sample scored above median value of HBV knowledge score. Similar finding was obtained in the study of Abeje and Azage<sup>14</sup> where 52% of the responders achieved scores greater than mean value. This is certainly below the common expectation that all HCWs should demonstrate good hepatitis B knowledge. These results highlighted considerable misunderstanding among health care professionals. In our sample of HCWs, higher values of hepatitis B knowledge score statistically significantly correlated with higher education level, younger age and less working experience. Additionally, meaningfully higher score is observed among HCWs who were vaccinated against HBV compared to the unvaccinated group, as well as among subgroup working at the operating room. Factors favouring better hepatitis B-related knowledge in our study were similar to those observed among HCWs in Greece<sup>15</sup>. Namely, Karaivazolou et al.<sup>15</sup> reported that higher education, occupation, and HBV vaccination were associated with hepatitis B knowledge score. Moreover, results from our study also introduced younger age and less working experience as factors that significantly contributed to higher HBV knowledge score. Previous studies reported conflicting results on age and HBV-related knowledge. Results of Adekanle et al.<sup>18</sup> supported our finding that younger age was associated with being more knowledgeable on HBV, while Afihene et al.<sup>19</sup> reported the opposite results suggesting that older age and more working experience were predictors of higher knowledge score. Higher HBV knowledge score in younger subgroup of our HCWs could be partially explained by more intensive hepatitis B-related education during the past decades in our medical schools.

Although most HCWs correctly identified that hepatitis B infection was caused by a virus, 18 (5.1%) participants misperceived this fact. Further analyses of characteristics of HCWs who answered incorrectly to this question showed that most (72.2%) belonged to non-medical subgroup of respondents, including administrative staff, sanitary workers and drivers. However, 2 medical technologists, 2 nurses, and even one physician were not knowledgeable regarding this basic question. In the study of Hassan et al.<sup>20</sup> was also shown that 7.3% of HCWs considered that the causing agent of hepatitis B was bacterium, while 6.5% considered that the agent was a parasite.

In our study, most HCWs showed good knowledge regarding various modes of HBV transmission, which is in agreement with the results from other authors<sup>13, 14, 18, 19</sup>. However, some authors suggested that significant proportion of HCWs were less knowledgeable regarding this matter, as they have indicated faecal-oral route and polluted water as possible modes of transmission<sup>14, 19</sup>. Furthermore, in our settings, poor knowledge concerning possible chronic complications of HBV infection has been observed. Specifically, large proportion of responders did not know that hepatitis B could lead to liver cirrhosis and liver cancer. It is clear that, in our group of HCWs, there is a knowledge gap surrounding comprehensive issues of hepatitis B. This was also supported by findings in other HCW cohorts<sup>18</sup>.

Majority of HCWs in our sample were aware of the availability of HBV vaccine, as well as that it was mandatory for all HCWs. However, 11 (3.1%) responders wrongly reported that HBV vaccine did not exist. This is in line with previous surveys, where in almost all HCW cohorts some participants incorrectly identify the absence of HBV vaccine<sup>13, 14, 18, 19</sup>. This finding certainly represents a major concern worldwide. Additionally, one-third of the HCWs in our survey demonstrated a lack of awareness on vaccination of newborns against HBV. In the Republic of Serbia, hepatitis B vaccine has been a part of the mandatory childhood immunization schedule for more than 10 years. Bearing in mind the fact that the first dose of HBV vaccine should be administered within the first days after birth, it is worrisome that such a large proportion of HCWs misperceive this practice. These data confirmed the need for additional efforts to improve hepatitis B vaccine awareness, promotion and implementation in our health-care community.

#### *Study limitations and strengths*

Some limitations of the present study need to be kept in mind in interpretation of our results. Firstly, this investigation was performed at one national clinical centre, thus selection bias cannot be excluded. Secondly, the cross-sectional design of our study captured the associations between several variables, but does not inherently allow us to make definite causality conclusions. Thirdly, an information bias should be acknowledged, because this study relies on self-reported data, which may be subject to over- or underestimation, potentially distorting results. Namely, data in our research were obtained through self-reported questionnaire. Although this approach has many advantages (low price, collecting data in a short period of time, etc.), it is dependent on sincerity of respondents which is generally linked to nature of questions. Finally, it is important to mention that comparison of results from different studies dealing with this issue is difficult due to heterogeneity in item structure questionnaire examining hepatitis B-related knowledge and different approaches to their scoring and interpretation. Apart from these limitations, this study has several strengths because it refers to a common public health problem and targets high risk cohort. Furthermore, it should be emphasized that such investigation was conducted for the first time in Serbia, and offer picture about hepatitis B-related awareness in our health care settings. We selected a representative sample of HCWs from a large referral health care facility. Thus, we hypothesize that the results of our study could be generalized to the total HCWs population of the country.

#### **Conclusion**

In summary, results of the present study suggest that the level of hepatitis B-related among HCWs is poor relative to some important aspects of HBV infection. Bearing in mind the fact that health care professionals are perceived as reliable source of information on hepatitis B, to both patients

and general public, it is essential that HCWs possess optimum knowledge on various aspects of this disease. Hence, well-planned educational campaigns and continuing medical education among this high-risk professional group should be intensified with primary focus on attitude change and behaviour modification to ensure hepatitis B-related safe and responsible health-care environment.

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## Cone-beam computed tomography study of tooth root and canal morphology of permanent molars in a Serbian population

Analiza morfologije korenova i kanala korenova stalnih molara primenom kompjuterizovane tomografije konusnog zraka u populaciji Srbije

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### Abstract

**Background/Aim.** For successful endodontic therapy, it is necessary to know root morphology. Therefore, the aim of our study was to analyze root canal morphology and root canal length of permanent molars in a Serbian population, using cone-beam computed tomography (CBCT). **Methods.** The study included a total of 305 maxillary molars, and 280 mandibular molars receiving cone-beam computed tomography examination and determined root numbers, canal morphology according to Vertucci classification, and canal lengths. Results were correlated with sex and tooth location in the jaw. **Results.** The mesiobuccal roots of first maxillary molars showed Vertucci type I in 45.7%, followed by type II in 29% of cases. For the second molar, Vertucci type I was found in 60.5% of cases in mesiobuccal canals. Palatal and distobuccal canals mostly presented Vertucci type I configuration. The mesial roots of mandibular molars had the highest frequency of two canals with Vertucci type IV as the most frequent for the first molar, and Vertucci type II for the second molar. Distal roots most commonly had one canal in both molars. Palatal canal length was the highest in maxillary first molars, with the mean value of 20.62 mm, while in second molars, the highest length value was for the mesiobuccal canal (20.09 mm). In both mandibular molars the mesial root canal was the longest one. Differences were found according to sex and tooth location in the jaw. **Conclusion.** Mesiobuccal roots of maxillary first molars had two canals; it was more frequently compared to second molars. Mesial roots of mandibular molars showed same frequency of two canals, and diversity in Vertucci types. Male patients tended to have higher complexity of root canal morphology compared to that of females. CBCT can improve understanding of the root canal morphology.

### Key words:

tooth root; molar; cone-beam computed tomography; serbia.

### Apstrakt

**Uvod/Cilj.** Za uspešnu endodontsku terapiju neophodno je poznavanje morfologije kanala korena zuba. Stoga je cilj naše studije bio da analiziramo morfologiju kanala i dužinu kanala korenova stalnih molara u populaciji Srbije, koristeći kompjuterizovanu tomografiju konusnog zraka (*cone-beam computed tomography* – CBCT). **Metode.** Studijom je obuhvaćeno ukupno 305 gornjih i 280 donjih molara snimljenih CBCT-om na kojima su analizirani broj korenova, morfologija kanala prema Vertučijevoj klasifikaciji i dužina kanala korenova. Procenjena je povezanost dobijenih rezultata sa polom i lokacijom zuba u vilici. **Rezultati.** Meziobukalni korenovi gornjeg prvog molara imali su tip I po Vertučiju u 45,7%, a tip II u 29% slučajeva. Kod gornjeg drugog molara, Vertučići tip I pronađen je u 60,5% slučajeva u meziobukalnim kanalima. Palatinalni i distobukalni kanali su uglavnom imali konfiguraciju kanala tipa I po Vertučiju. Mezijalni korenovi donjih molara najčešće su imali dva kanala, sa najvećom zastupljenošću Vertučići tipa IV u prvom, a Vertučići tipa II u drugom molaru. Distalni korenovi najčešće su imali jedan kanal u oba molara. Dužina palatinalnog kanala bila je najveća kod gornjih prvih molara, sa srednjom vrednošću od 20,26 mm, dok je kod drugih molara najveća bila vrednost meziobukalnih kanala (20,09 mm). Kod oba donja molara najduži su bili mezijalni kanali. Razlike su pronađene u odnosu na pol i stranu zuba u vilici. **Zaključak.** Meziobukalni korenovi gornjih prvih molara imali su češće dva kanala u poređenju sa drugim molarima. Mezijalni korenovi donjih molara pokazali su sličnu učestalost dva kanala i ranolikost u tipovima prema Vertučijevoj kasifikaciji. Kod osoba muškog pola zapažena je veća učestalost kompleksne morfologije kanala korenova u poređenju sa osobama ženskog pola. CBCT može poboljšati razumevanje morfologije kanala korenova.

### Ključne reči:

zub, korenski kanal; molari; tomografija, kompjuterizovana konusna; srbija.

## Introduction

The aim of endodontic therapy is to eliminate the inflamed tissue or infection from the root canal of the teeth, through mechanical shaping and chemical irrigation, followed by adequate obturation of the root canal<sup>1</sup>. In order to achieve a favourable outcome of endodontic therapy, knowledge of the root canal morphology is necessary<sup>2</sup>. Endodontic treatment of molar teeth presents a challenge due to the position of teeth in the jaw, the crown's morphological characteristics, the large number of roots and their complex canal system<sup>3</sup>. The first permanent molars are the first erupted teeth, and for this reason, they are often affected by caries, which can lead to pulpitis and a need for their endodontic treatment. In a number of cases, permanent molars are extracted, mostly due to the unsuccessful endodontic treatment<sup>2</sup>. The main reason for the failure in endodontic therapy is leaving the root canal untreated due to its complexity<sup>1,3</sup>. In addition, it is necessary to adequately determine the root canal length. Overtreating the canal or leaving it untreated, prior to the cementodentinal junction, can also be the cause of failure in endodontic therapy. Therefore it is important to have an overview of the expected, i.e. the average length of the root canal<sup>4-6</sup>.

It has been shown that the number of roots and root canals is different among populations, probably because these differences are genetically predetermined<sup>7</sup>. In the most cases, maxillary molars have three roots and three or four root canals. The frequency of the second mesiobuccal canal is around 50%<sup>8</sup>. Unlike the mesiobuccal canal, the morphology of the distobuccal and palatal canals is quite simpler, the distobuccal root has one canal in about 90% of cases, and palatal root in 99% of cases<sup>7,9</sup>. Mandibular molars have less complicated canal morphology than maxillary molars. Two roots are present in more than 87% of cases, and only 13% of mandibular molars have three roots<sup>1,10</sup>. Three roots are more present in Chinese, Native American, and Eskimo populations, while slightly simpler morphology is present in Caucasians<sup>7</sup>. The mesial root has two canals in 94%, while three canals are present in a small percentage. Differences in root canal morphology are claimed to be related to ethnicity as one root canal occurs more frequently in Caucasians<sup>7</sup>.

Morphological variations in the number of roots and canals are evaluated by various methods, and therefore the results vary depending on applied methods, as well as ethnicity, sex, age and side of the jaw<sup>11,12</sup>.

Digital dental radiography is required in endodontic therapy. Cone-beam computed tomography (CBCT) is a radiographic method that offers many benefits and possibilities, but it should not be routinely performed in endodontics, due to higher exposures to radiation. Mostly, patients have a CBCT scan taken for other reasons (interventions in oral surgery, orthodontics, and prosthodontics) and therefore can be used for endodontic evaluation as well<sup>6,7</sup>.

According to our knowledge, there are few studies that describe the root and root canal morphology in a Serbian population. The aim of this study was to evaluate the number of roots and root canal morphology, including canal length, of maxillary and mandibular first and second molars and

variations between sexes and side of the jaw. This study is part of a major research of the root morphology, which we conducted at the Faculty of Medical Sciences, University of Kragujevac, Serbia<sup>13,14</sup>.

## Methods

The research protocol was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia, and it was conducted in compliance with the Helsinki Declaration and Guidelines for Good Clinical Practice.

### Sample

The total study sample included images of 585 teeth, obtained from CBCT scans out of 192 patients from a pre-existing database. All CBCT images were made in Radiology Department, Faculty of Medical Sciences, University of Kragujevac, between October 25th, 2014 and October 25th, 2017. The scans were obtained using Orthophos XG 3D device (Sirona Dental Systems GmbH, Bensheim, Germany), with three-dimensional settings for recording, VOL1 or VOL1 HD, and a voxel size of 160  $\mu\text{m}$ ; the layer thickness was 0.16 mm with large Field of view (FOV). The reasons for CBCT scanning were different (prosthetic, surgical, orthodontic and endodontic).

The main image's inclusion criterion was the existence of at least one molar in the maxilla or mandible. Other inclusion criteria were following: tooth is fully visible; has completed root growth; has no radiographically visible periapical lesion; has no radiographically visible external or internal root resorption; is not treated endodontically and has no prosthetic restoration.

### Radiography and measurements

CBCT images were analyzed using a software GALAXIS v 1.9.4 (Sirona Dental Systems GmbH, Bensheim, Germany), in the axial, sagittal, and coronal sections. Observations were conducted at Philips LED monitor, sized 23-inch, with a resolution of 1,920  $\times$  1,080 pixels, in a room with dim lighting. Brightness and contrast were adjusted using a software program.

Teeth were classified into groups (maxillary first molar, maxillary second molar, mandibular first molar and mandibular second molar), and the following parameters were observed: the number of roots per tooth; the number of root canals per root; the root canal configuration according to Vertucci classification<sup>1</sup> (Figure 1 and Table 1); root canal lengths; position of the tooth – left or right side of the jaw; patient's sex.

Canal length was determined using a 3D approach as proposed by Tchorz et al.<sup>5</sup>. The length of the canal was considered to be the distance from the tip of the reference cusp to the apical foramen. The measurements were performed in the sagittal and axial plane, and the length was obtained by adding the distance between the reference



points. Since molars have root curvatures in many cases, they were marked as additional reference points located on

the axial cross-section and by adding the distances between them, the lengths of the molar root canals were obtained.

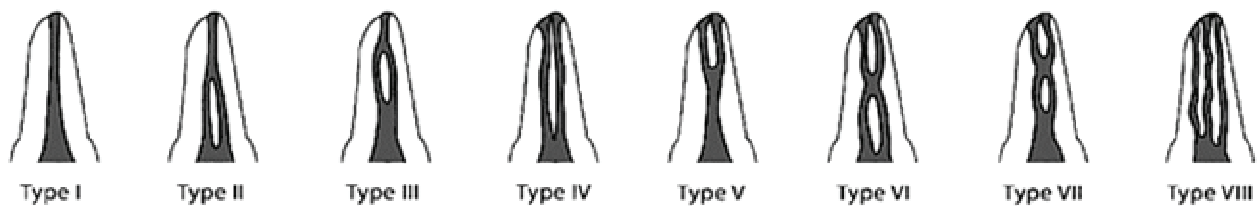


Fig. 1 – Diagrammatic representation of Vertucci root canal configurations.

Table 1

Descriptions of different root canal morphological types according to Vertucci

Type	Description
I	A single canal appears from the pulp chamber to the apex.
II	Two separate canals leave the pulp chamber but merge into one to the apex.
III	A single canal leaves the pulp chamber, divides into two within the root, and then merges to the apex.
IV	Two distinctly separate canals are present from the pulp chamber to the apex.
V	A single canal leaves the pulp chamber but divides into two with two apical foramina.
VI	Two separate canals leave the pulp chamber, join at the midpoint, and then divide again into separate canals with two separate apical foramina.
VII	One canal leaves the pulp chamber, divides and then rejoins within the root, and finally redivides into two separate canals with two separate apical foramina.
VIII	Three separate and distinct canals begin from the pulp chamber to the root apex.

### Statistics

Statistical data were analyzed using a commercial software package for statistics SPSS v 20.0 (SPSS Inc., Chicago, IL, USA). The frequencies and correlations of the values of the number of roots and the root canals and the root canal configuration according to Vertucci's classification were analyzed between the sexes and the side of the jaw on which the tooth is located using the  $\chi^2$  test and Fisher's exact test. Differences in the length of the root canal between the sexes and the jaw sides were analyzed using the Mann-Whitney *U*-test. The values of categorical variables are shown as percentages and numerical variables as the mean value and standard deviation (mean value  $\pm$  SD). The obtained *p* value less than 0.05 was considered as statistically significant.

### Results

In this study, mandibular and maxillary first and second molars were examined. We analyzed the number of roots and

canals, root canal length, and canal morphology according to Vertucci classification.

#### Maxillary molars – number of roots

A total of 305 maxillary molars were examined in this study.

The results for the number of roots are shown in Table 2. Of the total, 138 were maxillary first molars.

Within this subgroup, teeth with three roots were the most frequent finding (99.3%), while only one tooth was found with two roots (0.7%).

Within maxillary second molars, three roots were found in the majority of cases (90.4%), followed by two roots (5.4%), and one root (3.6%).

Four roots were found in only one case (0.6%).

There was a statistically significant difference in the number of roots according to sex ( $p = 0.008$ ) – one and two roots were more common in women (8.4%) than in men (1.2%).

Table 2

Number of roots of maxillary and mandibular molars

Tooth	Number (%) of roots			
	1 root	2 roots	3 roots	4 roots
Maxillary first molar	0 (0.0)	1 (0.7)	137 (99.3)	0 (0.0)
Maxillary second molar	6 (3.6)	9 (5.4)	151 (90.4)	1 (0.6)
Mandibular first molar	0 (0.0)	118 (100)	0 (0.0)	0 (0.0)
Mandibular second molar	4 (2.5)	158 (97.5)	0 (0.0)	0 (0.0)

### Number and morphology of root canals

Among distobuccal and palatal canals of maxillary molars, a single root canal, or Vertucci type I (100%), was found in the palatal root of maxillary first and second molars. Vertucci type I was the most frequent (97.1%) in distobuccal roots maxillary first molars, and the distobuccal roots of the maxillary second molars had one canal in all cases (100%).

The number of canals in the mesiobuccal roots of maxillary molars is shown in Table 3. The second mesiobuccal canal was present in 54.3% cases in the maxillary first molars, and 39.5% cases in the maxillary second molars (Figure 2). All types of Vertucci classification other than the

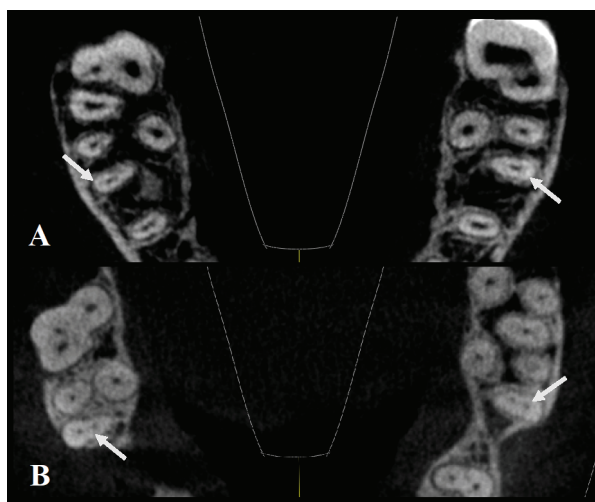
type I were considered as the presence of second mesiobuccal root canal. There was a statistically significant difference in the number of root canals according to sex for the maxillary first molar – one canal was more frequent in females (71.4%) than in males (28.6%), while two canals were more frequent in men (60.0%) than in women (40.0%). Vertucci classification of the mesiobuccal root canals of maxillary molars is shown in Table 4. There was a statistically significant difference in relation to sex ( $p = 0.001$ ), where type I was more frequent in women (71.4%), while type II (67.5%) was more frequent in men. In the maxillary second molar, there was no statistically significant difference in the root canal configuration between the sexes ( $p = 0.055$ ).

**Table 3**

**Number of canals in mesiobuccal roots of maxillary molars according to sex**

Tooth	Number of root canals	Sex, n (%)		Total	<i>p</i>
		male	female		
Maxillary first molar	1	18 (28.6)	45 (71.4)	63 (100)	0.003*
	2	45 (60.0)	30 (40.0)	75 (100)	
	Total	63	75	138	
Maxillary second molar	1	47 (46.5)	54 (53.5)	101 (100)	0.269
	2	37 (56.1)	29 (43.9)	66 (100)	
	Total	59	108	167	

\*Statistically significant difference using the Fisher's Exact Test.



**Fig. 2 – Axial cross-sections of the maxilla, arrows showing maxillary first molars with one canal (A), and two canals (B) bilaterally in mesiobuccal roots.**

**Table 4**

**Distribution of Vertucci classification in mesiobuccal roots of maxillary molars according to sex**

Tooth	Vertucci classification, n (%)						Total	<i>p</i>
	type I	type II	type III	type IV	type V	type VII		
Maxillary first molar								
Sex								
male	18 (28.6)	27 (67.5)	8 (66.7)	4 (33.3)	6 (54.5)	0 (0.0)	63	0.001*
female	45 (71.4)	13 (32.5)	4 (33.3)	8 (66.7)	5 (45.5)	0 (0.0)	75	
Total	63 (45.7)	40 (29.0)	12 (8.7)	12 (8.7)	11 (8.0)	0 (0.0)	138 (100)	
Maxillary second molar								
Sex								
male	47 (46.5)	22 (62.9)	4 (66.7)	4 (28.6)	7 (77.8)	0 (0.0)	84	0.055
female	54 (53.5)	13 (37.1)	2 (33.3)	10 (71.4)	2 (22.2)	2 (100)	83	
Total	101 (60.5)	35 (21.0)	6 (3.6)	14 (8.4)	9 (5.4)	2 (1.2)	167 (100)	

\*Statistically significant difference using the Pearson  $\chi^2$ -test.

### Mandibular molars – number of roots

In the analysis of 280 mandibular molars on the CBCT, all mandibular first molars were two-rooted (100%), while among mandibular second molars, two roots were present in the majority of cases (Table 2). There was no statistically significant difference in relation to sex or jaw side.

### Number and morphology of root canals

Of examined 118 mandibular first molars, the mesial root had two root canals in 94.9%, and one canal in 5.1% of cases. The distal root had one root canal in 89.8%, while two root canals were present in 10.2% of cases. Out of total 162

mandibular second molars, the mesial root had two canals in 85.2%, and one canal in 14.8% cases. One canal was the most prevalent in the distal root of the mandibular second molar (98.7%), while two canals were founded in 1.3% of cases (Figure 3).

Analysis of the distal root canal morphology of the mandibular first molar showed the highest frequency of Vertucci type I (89.8%), followed by Vertucci type II and type III (5.1% both). In the distal root of the mandibular second molar, the most common was Vertucci type I (98.7%), followed by Vertucci type II (1.3%). The differences between sexes or jaw sides, in canal configurations of the mesial root of the mandibular first and second molars, were not statistically significant (Table 5).

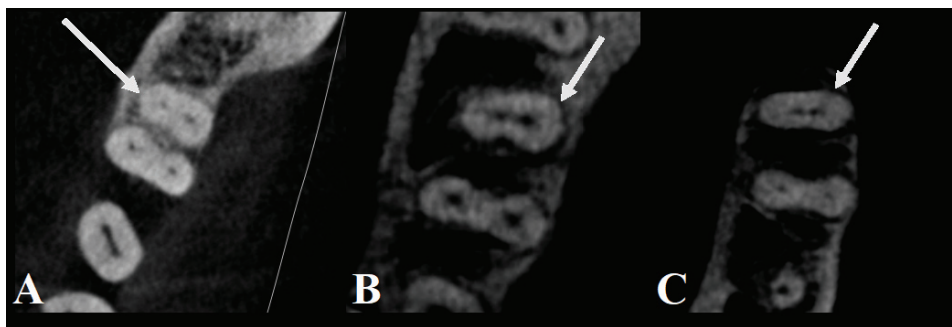


Fig. 3 – Axial cross-sections of mandibular first (A, B) and second (C) molars, arrows showing two canals in distal roots.

Table 5

Distribution of Vertucci classification in mesial roots of mandibular molars according to sex

Tooth	Vertucci classification, n (%)				Total	p
	Type I	Type II	Type III	Type IV		
Mandibular first molar						
Sex						
male	4 (66.7)	26 (61.9)	0 (0.0)	28 (40.0)	58	0.055
female	2 (33.3)	16 (38.1)	0 (0.0)	42 (60.0)	60	
Total	6 (5.1)	42 (35.6)	0 (0.0)	70 (59.3)	118 (100)	
Mandibular second molar						
Sex						
male	14 (58.3)	42 (63.6)	4 (40.0)	36 (58.1)	96	0.551
female	10 (41.7)	24 (36.4)	6 (60.0)	26 (41.9)	66	
Total	24 (14.8)	66 (40.7)	10 (6.2)	62 (38.3)	162 (100)	

### Canal length of maxillary and mandibular molars

A total of 585 molars were measured, and the results for canal length are shown in Table 6. The mean length of the palatal canal for the maxillary first molar was 20.62 mm, and for the maxillary second molar it was 18.86 mm. The mean value of the mesiobuccal canal length was 19.30 mm among first maxillary molars and 20.09 mm among second maxillary molars. The average length of the distobuccal canal was 19.08 mm among first maxillary molars, and 19.11 mm among maxillary second molars.

Mesial root canals of mandibular molars had the average length of 20.72 mm for the first molar, and 20.06 mm for the second molar. The distal root canal of mandibular molars had an average length of 20.05 mm among first

mandibular molars, and 19.64 mm among second mandibular molars.

The differences in canal lengths were compared between sexes and jaw side. For the maxillary first molar, there was a statistically significant difference in relations to sex – the palatal, mesiobuccal and distobuccal canals of the maxillary first molar were found to be longer in the males. Among second maxillary molars, a statistically significant difference ( $p=0.021$ ) was found in the length of the distobuccal canal, which was longer in men. In relation to the jaw side, there was a statistically significant difference in mesiobuccal ( $p=0.035$ ) and palatal canal ( $p=0.045$ ). The mean lengths of the mesiobuccal and palatal canal were higher on the left side of the jaw. The difference in mean length of distobuccal root canals according to sex was

statistically significant ( $p = 0.021$ ). The difference was significant for mesial root canal of the first mandibular molar relative to the side ( $p = 0.034$ ). For the mandibular second

molar, statistical significance was found for both mesial and distal canal according to sex.

**Table 6****Root canal lengths of maxillary and mandibular molar roots according to sex and jaw side**

Tooth	Range, mm		Mean (SD), mm		<i>p</i>	Mean (SD), mm			<i>p</i>
	min.	max.	male	female		right side	left side	Total	
Maxillary first molar									
Root									
mesiobuccal	16.18	23.41	19.67 (1.34)	18.72 (1.24)	< 0.001*	18.84(1.34)	19.45(1.38)	19.30 (1.42)	0.035*
distobuccal	14.24	21.69	19.26(1.47)	18.52(1.60)	0.012*	18.72(1.91)	18.99(1.17)	19.08 (1.59)	0.941
palatal	18.02	22.72	20.93 (0.93)	20.20(1.03)	< 0.001*	20.30(1.14)	20.75(0.90)	20.62 (1.04)	0.045*
Maxillary second molar									
Root									
mesiobuccal	16.13	25.11	19.25 (1.52)	19.06(1.42)	0.379	19.06(1.42)	19.21(1.51)	20.09(1.37)	0.642
distobuccal	15.71	23.25	18.89(1.32)	18.54(1.42)	0.021*	18.71(1.48)	18.73(1.31)	19.11(1.44)	0.472
palatal	16.10	23.16	20.18(1.64)	19.76(1.04)	0.060	19.77(1.37)	20.11(1.42)	18.86(1.27)	0.071
Mandibular first molar									
Root									
mesial	17.89	25.50	20.73 (1.81)	20.72(1.42)	0.763	20.76(1.49)	20.69(1.73)	20.72(1.61)	0.643
distal	17.57	23.40	19.96(1.63)	20.16(1.04)	0.182	20.35(1.48)	19.77(1.18)	20.05(1.36)	0.034*
Mandibular second molar									
Root									
mesial	17.23	23.78	20.47(1.62)	19.44(0.91)	< 0.001*	20.16(1.54)	19.95(1.38)	20.06(1.46)	0.585
distal	17.20	23.63	19.98(1.44)	19.11(1.04)	< 0.001*	19.69(1.55)	19.58(1.14)	19.64(1.37)	0.906

SD – standard deviation; \*Statistically significant difference using the Mann-Whitney *U*-test.

## Discussion

In Europe, CBCT, as a radiographic technique designed specifically for hard tissues of the maxillofacial region was introduced in dental practice in 1998<sup>15</sup>. Today, it is most commonly used in the fields of oral surgery, implantology, and orthodontics, but also in the field of endodontics for a three-dimensional analysis of the external and internal tooth morphology<sup>12</sup>. Other than CBCT, a variety of different techniques are used to analyze the root and root canal morphology such as classical radiography, an electronic microscope, micro-CT and cleaning and staining technique, which is considered the gold standard. In the terms of the precision, CBCT shows the same level as cleaning and staining technique<sup>16</sup>. CBCT provides high precision, minimal distortion and 3-dimensional (3D) image projection<sup>8</sup>.

Among maxillary first molars, we obtained the highest prevalence of three roots and a small percentage of single rooted first molars (0.7%) as reported by other studies<sup>2, 5, 8, 17, 18</sup>. The diversity of root numbers in maxillary first molars was not found. The Alrahabi et al.<sup>19</sup> showed a slightly smaller percentage of molars with three roots, while Barbizam et al.<sup>20</sup> showed molars with four and five roots in their population, but still in a very small percentage.

Canal configuration of palatal root showed no variations, with one canal in all cases, as shown by other studies in diverse populations<sup>8, 11, 18, 19</sup>. In the distobuccal root, Vertucci type I was the most prevalent with a scarce variation in canal morphology. Similar results were shown by Ghoncheh et al.<sup>18</sup> for Iranian population (97.33%), as well as others<sup>2, 8, 11, 19, 21</sup>.

Unlike palatal and distobuccal, the mesiobuccal root showed diversity in the number of canals and canal morphology. In our study, two mesiobuccal canals were present in more than half of the examined first molars, with Vertucci type II as the most frequent (28.98%). Previous studies have also shown the highest percentage of two mesiobuccal canals<sup>2, 8, 11, 22, 23</sup>. Vertucci type II, as the most prevalent complex configuration, was shown in a study of Ratanajirasut et al.<sup>8</sup> (28.98%) for Thai population and, with a slightly lower percentage (23.2%), in a study of Pérez-Heredia et al.<sup>24</sup> for Spanish population. Vertucci type III and type IV followed with the same frequency (8.69%). Approximate results for type IV were shown in a study conducted in Turkey<sup>25</sup>, while studies conducted in India and Korea<sup>23, 26</sup> reported type IV in a much larger percentage (40.65% and 38.6%, respectively). We demonstrated higher frequency of two mesiobuccal canals in males than females. These results were in accordance with our previous study of premolar teeth<sup>14</sup>. For maxillary molars, our results were similar to findings in Thai population,<sup>8</sup> where 70.9% of males had complex canal configurations.

Unlike the maxillary first molar, the second molars showed a slightly higher diversity in the number of roots. In our study, most maxillary second molars had three roots (90.4%), as in a study of Ratanajirasut et al.<sup>8</sup> for Thai population (78.1%) and other studies<sup>18, 23, 24, 26</sup>. Two roots were much less frequent (5.4%), which coincides with the results for Iranian and Indian populations<sup>18, 26</sup>. Single-rooted maxillary second molars were considerably less frequent, but the recorded percentage was higher compared with first molars. Similar results were found for different populations in other studies<sup>8, 18, 23, 24, 26</sup>.

All the palatal and distobuccal roots of maxillary second molars presented Vertucci type I configuration. These findings are consistent with previous results for Thai<sup>8</sup>, Korean<sup>23</sup> and Spanish population<sup>24</sup>. More diversity in root canal configurations was found for Iranian and Indian populations<sup>18,26</sup>, which can be attributed to our study sample size.

In the mesiobuccal root of maxillary second molars, two canals were present in 39.5%. The most frequent was the Vertucci type I configuration as previously reported<sup>8,18,23,24,26</sup>. Our study indicated that the second molars had most frequently Vertucci type II, which is similar to a study in European population<sup>24</sup> and several studies conducted in Asia<sup>8,23</sup>. Other studies of Asian population, made by Ghoncheh et al.<sup>18</sup> and Neelakantan et al.<sup>26</sup> showed the second highest prevalence of Vertucci type IV. In our study type IV was presented as the third most frequent. These differences could be due to population's ethnical origin or a result of different sample's size. Our findings presented Vertucci type VII in 2 cases, but no cases with type VI or VIII, unlike previous studies<sup>8,18,23,24,26</sup>.

These results could be attributed to a difficulty to differentiate types with such complexity. Unlike in first molars, results for prevalence of two mesiobuccal canals in the maxillary second molar showed no statistical difference between sexes, although it was noticeable that two root canals were more frequent in males. Identical results were shown previously<sup>8,23</sup>.

Previous studies have shown that the first mandibular molars have two roots, predominantly. A study in the white population<sup>2</sup> showed that all analyzed molars had two roots, as presented in the study we conducted. Similar results were found in studies conducted in Spain<sup>24</sup>, and Belgium and Chile<sup>27</sup>, while studies conducted in Korea<sup>5</sup> and Thailand<sup>28</sup> showed a lower percentage of two roots (87.3% and 73.5%, respectively).

As expected, the distal root presented a simpler root canal morphology than mesial. Vertucci type I was the most frequent, which coincides with the results of studies conducted in Spain and Belgium<sup>24,27</sup>. Vertucci types II and III were found in a low percentage, as in other studies<sup>5,24,29</sup>.

Unlike distal root of mandibular molars, the mesial root presents a challenge in endodontic therapy. Our findings showed that in most cases, the mesial root had two canals, with the highest frequency of Vertucci type IV, which was confirmed in a study on white population<sup>2</sup>, but also on populations of Thailand<sup>5</sup>, Burma<sup>10</sup> and Uganda<sup>29</sup>. Vertucci type II was the second most frequent in our population, which was also reported for diverse populations<sup>2,5,24,29</sup>. Unlike in our population, where the frequency of type III and type V was quite low, a study conducted in Chile and Belgium showed the opposite<sup>27</sup>.

The mandibular second molar did not show large variations in root numbers, with two-rooted molars mostly presented, which was previously reported as well<sup>2,24,27</sup>. The number of canals in mesial root showed similarity to mandibular first molar, while distal root was presented with one root canal in almost all cases. Previous reports showed similar results<sup>2,10,25,27,29</sup>.

Our findings showed Vertucci type II to be the most common finding in the mesial root, followed by type IV. Studies conducted in Thailand<sup>5</sup>, Burma<sup>10</sup> and Uganda<sup>29</sup> showed the highest frequency of Vertucci type IV. Unlike our findings for mandibular first molar, a low frequency of Vertucci type III was presented for the second molar. Contrary to ours, populations of Belgium and Chile showed a higher prevalence of this type<sup>29</sup>.

Our findings did not show any statistical differences in canal complexity between sexes for both mandibular molars. Lack of sex differences was previously reported by Kim et al.<sup>28</sup>.

Besides knowledge of the root canal morphology and the number of roots, for the success of endodontic therapy, knowledge of adequate canal root length is also required. CBCT has found its application in endodontics in the analysis of canal morphology and finding an unexpected number of canals, and gradually it finds application in measuring the length of the root canal as well. For example, in North Korea, the most common non-surgical procedure for which CBCT is used, is measuring the length of the canal. A 3D approach in measuring the length of the root canal has shown to give little variations (0.22–0.42 mm) from the actual canal length<sup>6</sup>. Liang et al.<sup>30</sup> assessed CBCT validity in the measurement of canal length compared to the gold standard, electro-odontometry. They showed that the absolute difference between these two methods was ranging from 0.44 to 0.59 mm, and stated that the difficulty to determine the working length of the molar canals due to the large curvature of the roots must be considered<sup>30</sup>. It may not be necessary to use CBCT only for determining the length of the root canal, but if patients have a CBCT image for some other indication, we might have a possibility to get an insight into the expected working lengths. Surely, in combination with electro-odontometry, in everyday practice, we can increase the accuracy of the measured working length and success of endodontic treatment.

According to our knowledge, there are not many studies dealing with this topic, but for example, in the study of Rodríguez-Niklitschek et al.<sup>31</sup> the length of the second maxillary and mandibular molars were measured by the electro-odontometry method. The average lengths of mesiobuccal and distobuccal canals of the second maxillary molars were similar to those in our study, except for the mean length of the palatal canal that was smaller in our population than in Chile<sup>31</sup>. In the Chilean study, there was no statistically significant difference according to sex, opposed to our study where we found that distal canals were longer in males. The average length of the canals of the second mandibular molars in our population was similar to those in the study of Rodríguez-Niklitschek et al.<sup>31</sup> root canals were significantly longer in male subjects in both studies.

To our knowledge, side asymmetry in tooth lengths, and possible causes of such a finding had not been previously reported. Keles et al.<sup>32</sup> demonstrated asymmetries in the facial area and stated that differences were related to handedness, where right-handed persons had larger left facial area compared to left-handed and *vice versa*. Our study lacks in data regarding handedness.

## Conclusion

The results of this study founded that three roots were the most frequent in both maxillary first and second molars, while the canal morphology was different. The two mesio-buccal canals of the maxillary first molar were present in more than half of the examined teeth, while the frequency was lower for the maxillary second molar. As expected, palatal and distobuccal roots did not have complex canal morphology, contrary to mesiobuccal root canal. Mandibular molars also did not show big variations in the number of roots – two roots were most frequently present in both molars. The distal root had simple canal morphology with one root canal in the majority of cases. Also, the mesial roots of both molars showed two canals in the most of cases, but the frequency of Vertucci types were different – type IV was

most frequently present in the mesial root of the mandibular first molar, while in the second molar, type II was most frequently present.

The palatal canal was the longest in maxillary first molars, followed by mesiobuccal and distobuccal root canals. Mesial canals of mandibular molars were longer than distal root canals. The mean root canal lengths in both maxillary and mandibular molars were bigger in males.

In relation to the jaw side, there was a statistically significant difference in the length of the mesiobuccal and palatal root canals, which were found to be longer on the left side of the jaw. The results of our study showed, that the attention should be paid during endodontic treatment because there are differences not only in root canal morphology, but also in the length of the root canals between populations, sexes and side of the jaw.

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# Evolution of concomitant moderate and moderate to severe functional mitral regurgitation following aortic valve surgery for severe aortic stenosis

Evolicija istovremene umerene i umerene do teške funkcionalne mitralne regurgitacije nakon operacije teške stenozе aortnog zaliska

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## Abstract

**Background/Aim.** Functional mitral regurgitation (FMR) is a common entity in patients with aortic stenosis (AS) undergoing aortic valve replacement (AVR). The aim of this study was to examine evolution of moderate and moderate to severe FMR after an isolated AVR, to identify prognostic indicators for persistent MR postoperatively, and to offer the recommendation regarding surgical intervention for moderate and moderate to severe FMR at the time of AVR for AS. **Methods.** We retrospectively reviewed 39 consecutive patients with moderate and moderate to severe FMR at the time of isolated AVR from January 2007 to December 2013. We collected preoperative and postoperative echocardiographic data to determine the evolution of FMR after AVR. Patients were divided into the persistent ( $n = 14$ ) and improved FMR group ( $n = 25$ ). Secondary division was into the prosthesis-patient mismatch (PPM,  $n = 7$ ) and non prosthesis-patient mismatch group (non PPM,  $n = 32$  patients). Late follow-up echocardiography was completed in 100% (39/39) of patients. **Results.** FMR improved postoperatively ( $MR < 2+$ ) in 64% (25/39) of patients, while 36% (14/39) of patients had persistent  $MR \geq 2$ . In

comparison to the persistent group, the patient with improved FMR had significant decrease in the left ventricular end-diastolic diameter, left ventricular end-systolic diameter, posterior wall and septum thickness postoperatively. The same indicators of reverse remodeling were found in the non PPM group in comparison to the PPM group. The incidence of postoperative FMR improvement was higher in the non PPM group (65.6%,  $p = 0.001$ ) in comparison to the PPM group (42.9%,  $p = 0.125$ ). The mean follow-up duration was  $39.5 \pm 23.5$  months. **Conclusion.** In accordance with previous studies, this study also showed improvement in FMR following AVR surgery. Improvement in MR degree was associated with echocardiographic parameters of reverse left ventricular remodeling. Conservative approach is advisable in patients with moderate and moderate to severe FMR, believing that repair or replacement is unnecessary at the time of AVR for severe AS. PPM could prevent downgrading of FMR, stressing out the importance of choosing the prosthesis of adequate size.

## Key words:

mitral valve insufficiency; aortic valve stenosis; cardiovascular surgical procedures; postoperative period.

## Apstrakt

**Uvod/Cilj.** Funkcionalna mitralna regurgitacija (FMR) je čest entitet kod bolesnika sa stenozom aortnog zaliska (AS), kojima je indikovana zamena aortnog zaliska (ZAZ). Cilj ove studije bio je da ispita evoluciju umerene i umerene do teške FMR nakon izolovane ZAZ, da identifikuje prognostičke indikatore za perzistentnu MR posle operacije, i da ponudi preporuku za operaciju umerene i umerene do teške FMR u vreme ZAZ zbog AS. **Metode.** Retrospektivno je ispitano 39 konsektivnih bolesnika sa umerenom i umerenom do teškom FMR u trenutku izolovane ZAZ, od

januara 2007. do decembra 2013. Godine. Priključeni su preoperativni i postoperativni ehokardiografski podaci da bi se analizirala evolucija FMR nakon ZAZ. Bolesnici su bili podeljeni na perzistentnu grupu ( $n = 14$ ) i grupu sa smanjenom FMR ( $n = 25$ ). Naknadna podela je bila na grupu sa bolesnik-proteza diskrepancom (PPD,  $n = 7$ ) i *non* bolesnik-proteza diskrepancom (*non* PPD,  $n = 32$ ). Udaljeno ehokardiografsko praćenje je kompletirano kod 100% (39/39) bolesnika. **Rezultati.** Kod 64% (25/39) bolesnika, FMR je bila smanjena postoperativno ( $MR < 2+$ ), a kod 36% (14/39) bolesnika je perzistirala ( $MR \geq 2+$ ). Za razliku od perzistentne grupe, bolesnici sa smanjenom FMR su nakon



operacije imali značajnu redukciju dijametra leve komore na kraju diastole, dijametra leve komore na kraju sistole, debljine zadnjeg zida i septuma. Isti indikatori reverznog remodelovanja su nađeni i kod *non* PPD grupe u poređenju sa PPD grupom. Incidenca smanjenja postoperativne FMR je bila veća kod *non* PPD grupe (65,6%,  $p = 0,001$ ) u odnosu na PPD grupu (42,9%,  $p = 0,125$ ). Srednje vreme ehokardiografskog praćenja bolesnika je bilo  $39,5 \pm 23,5$  meseci. **Zaključak.** U skladu sa prethodnim studijama, ova studija takođe pokazuje poboljšanje FMR nakon ZAZ. Smanjenje stepena MR je udruženo sa ehokardiografskim parametrima

reverznog remodelovanja leve komore. Preporučuje se konzervativan pristup bolesnicima sa umerenom i umereno do teškom FMR, sa stavom da je rekonstrukcija ili zamena nepotrebna u trenutku ZAZ zbog AS. PPD može da spreči smanjenje FMR, te se naglašava značaj izbora proteze odgovarajuće veličine.

#### Ključne reči:

**zalistak, mitralni, insuficijencija; zalistak, aortni, stenoza; hirurgija, kardiovaskularna, procedure; postoperativni period.**

## Introduction

Mitral regurgitation (MR) is a frequent coexisting dysfunction in patients with severe aortic valve stenosis (AS). Some degree of MR is found in as much as 61% to 90% of patients undergoing aortic valve replacement (AVR) for AS<sup>1</sup>.

Functional mitral regurgitation (FMR) has been reported in as high as 75% of patients who undergo AVR<sup>2</sup>.

According to the guidelines, mitral valve (MV) surgery is reasonable for patients with chronic severe secondary MR who are undergoing AVR, and MV repair may be considered for patients with chronic moderate secondary MR who are undergoing other cardiac surgery<sup>3</sup>. As long as there are no morphological leaflet abnormalities, mitral annulus dilatation or marked abnormalities of left ventricular (LV) geometry, surgical intervention on MV is in general not necessary and non-severe secondary MR usually improves after aortic valve treatment<sup>4</sup>.

Although mitral valve surgery at the time of AVR may increase perioperative mortality and morbidity, the effect of residual MR on survival, quality of life and development of heart failure is important too.

Data from the Society for Thoracic Surgery (STS) database (2002–2006) reported an overall unadjusted mortality of 3.2% following AVR in a population of 6,292 patients at 809 centers worldwide. Double valve replacement is, as expected, associated with a significantly higher operative risk with a postoperative mortality at 11–12%, emphasizing that careful patient selection is imperative<sup>5</sup>. Gillinov et al.<sup>5</sup> suggested that MV repair during double valve surgery might be beneficial compared to mitral replacement, with a long-term reduction in mortality (34% versus 46%), without increased perioperative mortality. Talwar et al.<sup>6</sup> reported that MV repair with AVR provided significantly better event-free survival than double valve replacement.

Schubert et al.<sup>7</sup> emphasized that in patients whose MR improved postoperatively, 5-year survival was 73.5%, compared with 65.4% in patients whose MR did not improve ( $p = 0.06$ ). Survival was worse in patients whose MR worsened (46.7%;  $p < 0.01$ ). Barreiro et al.<sup>8</sup> found that patients with persistent or worsening MR after AVR tended to have a lower 5-year survival. Vanden Eynden et al.<sup>9</sup> found a trend towards better 10-year survival in patients with improved postoperative MR.

The aim of this study was to examine evolution of moderate and moderate to severe functional MR after isolated AVR, and to identify prognostic indicators for persistent MR postoperatively. Also, we intended to set a recommendation – should FMR be operated simultaneously with AVR.

## Methods

### Patient selection

From January 2007 to December 2013, a total of 1,104 patients underwent isolated AVR for severe AS, at the Dedinje Cardiovascular Institute, Belgrade, Serbia. From this group we excluded patients with: morphologic abnormalities of the mitral apparatus, calcification or fibrosis of leaflets, chordae rupture, leaflet prolaps, significant coronary artery stenosis, aortic disease, previous open heart procedures, and congenital disease. After these exclusions, 39 patients were enrolled in the study.

We conducted a retrospective study of 39 consecutive patients. Patient's demographics, clinical characteristics and preoperative and postoperative echocardiographic data were collected in a retrospective manner for the entire cohort.

Primarily, patients were stratified into two groups based on improvement or no improvement of their FMR at the last follow-up echocardiogram after AVR. Persistent FMR group (14 patients, 36%) remained in moderate and moderate to severe grade (2+ and 3+) after AVR. Improved FMR group (25 patients, 64%) had a reduction in MR grade (less than 2+) after AVR.

Secondarily, we formed additional two groups of patients, based on the value of indexed Effective Orifice Area (EOAi), of the implanted aortic prosthesis. The EOA was derived from the manufacturer's published values of projected *in vivo* EOA. This value was indexed to body surface area to yield the indexed effective orifice area of the valve. Prosthesis-patient mismatch group (PPM) (7 patients, 18%) had  $EOAi \leq 0.85 \text{ cm}^2/\text{m}^2$ . Non prosthesis-patient mismatch group (non PPM) (32 patients, 82%) had  $EOAi > 0.85 \text{ cm}^2/\text{m}^2$ .

The values, distributions and frequencies of preoperative and postoperative variables between groups were compared, to determine if any significant differences were associated with postoperative improvement or worsening of functional MR.

AVR was performed using mechanical St. Jude Medical™ Hemodynamic Plus Aortic Valve in 36 patients, and St. Jude Medical™ Biocor™ Pericardial Stented Tissue Valve in 3 patients.

Our study was approved by the Institutional Review Board of the Dedinje Cardiovascular Institute, with a waiver of the requirement for an individual patient consent.

#### *Echocardiography and grading of mitral regurgitation*

All studied patients went through preoperative and postoperative transthoracic echocardiography, a complete M-mode, bidimensional and Doppler echocardiographic assessment according to the European Association of Echocardiography and the American Society of Echocardiography guidelines<sup>10</sup>.

The diagnosis of a severe AV stenosis was established by preoperative echocardiography.

Grading of MR was as follows: 0 for no regurgitation, 0.5 for trace, 1+ for mild, 2+ for moderate, 3+ for moderate-severe, and 4+ for severe, as defined by the American Society of Echocardiography. Grading was done by preoperative transthoracic echocardiography<sup>7,10</sup>.

#### *Follow-up*

Postoperative echocardiography was routinely performed before discharge. Late follow-up echocardiograms were obtained on the patients at variable intervals and at the discretion of the patients' individual cardiologists. Late follow-

up echocardiographic data were obtained for 100% (39/39) of patients. We use the latest echocardiography findings for the comparison.

#### *Statistical Analysis*

All data are expressed as mean  $\pm$  standard deviation (SD) or as absolute values and percentages. Statistical analysis was done using the Student's independent *t*-test, paired-samples *t*-test,  $\chi^2$  or Fisher's exact test and Wilcoxon's signed-rank test. Statistical significance was defined as a two-tailed *p* value less than 0.05. SPSS for Windows, version 20.0 (SPSS Inc, Chicago, IL) was used for statistical analysis.

#### **Results**

##### *Improved FMR group versus persistent FMR group*

In the cohort of 39 patients, 25 improved FMR and 14 had persistent FMR postoperatively. There was no difference in the preoperative demographic, clinical and echocardiographic parameters between groups, except left ventricle ejection fraction (LVEF). Persistent FMR patients had lower preoperative values of LVEF ( $41.79 \pm 17.93\%$  vs.  $53.6 \pm 14.76\%$ ,  $p = 0.033$ ) (Table 1). The mean late follow-up duration was  $42.64 \pm 20.72$  months in the persistent FMR group, and  $37.72 \pm 25.13$  months in the improved FMR group ( $p = 0.537$ ).

**Table 1**

**Comparison of the baseline patient characteristics in respect to function mitral regurgitation (FMR) persistence**

Variables	Persistent FMR (n = 14)	Improved FMR (n = 25)	<i>p</i> value
Age (years), mean $\pm$ SD	59.36 $\pm$ 12.57	64.08 $\pm$ 9.45	0.192
Female sex, n (%)	6 (42.9)	17 (68)	0.126
BSA (m <sup>2</sup> ), mean $\pm$ SD	1.79 $\pm$ 0.17	1.84 $\pm$ 0.16	0.363
BMI (kg/m <sup>2</sup> ), mean $\pm$ SD	25.17 $\pm$ 3.61	26.60 $\pm$ 5.27	0.373
Atrial fibrillation, n (%)	1 (7.1)	5 (20)	0.391
NYHA functional class, n (%)			
II	13 (92.9)	24 (96)	1.000
III	1 (7.1)	1 (4)	
TAV, n (%)	13 (92.9)	22 (88)	1.000
AVA (cm <sup>2</sup> ), mean $\pm$ SD	0.69 $\pm$ 0.18	0.61 $\pm$ 0.17	0.200
Peak gradient (mmHg), mean $\pm$ SD	90.57 $\pm$ 32.73	109.08 $\pm$ 29.07	0.076
Mean gradient (mmHg), mean $\pm$ SD	54.86 $\pm$ 20.21	70.72 $\pm$ 25.44	0.053
LVEDD (mm), mean $\pm$ SD	57.86 $\pm$ 11.45	52.40 $\pm$ 5.92	0.115
LVESD (mm), mean $\pm$ SD	41.50 $\pm$ 12.46	35.20 $\pm$ 7.19	0.052
Septum thickness (mm), mean $\pm$ SD	11.86 $\pm$ 1.79	12.04 $\pm$ 1.88	0.769
Posterior wall thickness (mm), mean $\pm$ SD	11.86 $\pm$ 1.56	11.72 $\pm$ 1.46	0.785
LA (mm), mean $\pm$ SD	41.86 $\pm$ 4.80	43.00 $\pm$ 4.55	0.465
LVEF (%), mean $\pm$ SD	41.79 $\pm$ 17.93	53.6 $\pm$ 14.76	0.033
TR grade, n (%)			
0	7 (50)	14 (56)	
I	3 (21.4)	5 (20)	1.000
II	4 (28.6)	6 (24)	
RVSP (mmHg), mean $\pm$ SD	47.33 $\pm$ 8.45	44.00 $\pm$ 10.95	0.529
EAOi (cm <sup>2</sup> /m <sup>2</sup> ), mean $\pm$ SD	1.15 $\pm$ 0.39	1.13 $\pm$ 0.29	0.846

**Note:** Results are presented as mean  $\pm$  standard deviation or as absolute values (percentages).

**BSA** – body surface area; **BMI** – body mass index; **TAV** – tricuspid aortic valve; **AVA** – aortic valve area; **LVEDD** – left ventricular end-diastolic diameter; **LVESD** – left ventricular end-systolic diameter; **LA** – left atrial diameter; **LVEF** – left ventricular ejection fraction; **TR** – tricuspid regurgitation; **RVSP** – right ventricular systolic pressure; **EAOi** – effective orifice area index.

*Persistent FMR group – before versus after AVR*

Following aortic valve replacement, peak and mean transvalvular pressure gradients reduced significantly. Gradients measured across aortic prostheses were significantly lower than gradients measured across severely stenosed native valve. The remaining echocardiographic parameters did not change significantly (Table 2).

*Improved FMR group – before versus after AVR*

Following aortic valve replacement, peak and mean transvalvular pressure gradients reduced significantly. Gradients measured across aortic prostheses were significantly lower than gradients measured across severely stenosed native valve. In addition, left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD),

septum thickness, left ventricular posterior wall thickness, also reduced significantly (Table 3). In other words, there was a significant reverse remodeling of the LV in this group of patients.

*PPM group versus non PPM group*

In the cohort of 39 patients, 32 were in the non PPM group ( $EOAi > 0.85 \text{ cm}^2/\text{m}^2$ ), and 7 were in the PPM group ( $EOAi \leq 0.85 \text{ cm}^2/\text{m}^2$ ). There was no difference in the preoperative demographic, clinical and echocardiographic parameters between groups, except the patients' age. The PPM patients were older than the non PPM ones ( $71.00 \pm 6.16$  vs.  $60.50 \pm 10.68$ , respectively;  $p=0.017$ ) (Table 4). The mean late follow-up duration was  $36.47 \pm 20.12$  months in the non PPM group, and  $53.29 \pm 33.63$  months in the PPM group ( $p = 0.086$ ).

**Table 2**  
Changes in echocardiographic data after aortic valve replacement (AVR) in the persistent functional mitral regurgitation (FMR) group

Variables	Preoperative	Late follow-up	<i>p</i> value
Peak gradient (mmHg), mean $\pm$ SD	90.57 $\pm$ 32.73	29.14 $\pm$ 8.74	0.001
Mean gradient (mmHg), mean $\pm$ SD	54.86 $\pm$ 20.21	15.93 $\pm$ 5.18	0.001
LVEDD (mm), mean $\pm$ SD	57.62 $\pm$ 11.88	57.69 $\pm$ 11.82	0.861
LVESD (mm), mean $\pm$ SD	41.31 $\pm$ 12.95	40.92 $\pm$ 13.87	0.687
Septum thickness (mm), mean $\pm$ SD	11.92 $\pm$ 1.85	11.46 $\pm$ 1.94	0.190
Posterior wall thickness (mm), mean $\pm$ SD	11.92 $\pm$ 1.61	11.46 $\pm$ 1.90	0.111
LA (mm), mean $\pm$ SD	41.77 $\pm$ 4.99	42.15 $\pm$ 4.69	0.457
LVEF (%), mean $\pm$ SD	41.92 $\pm$ 18.66	41.62 $\pm$ 15.75	0.824
TR grade, n (%)			
0	7 (50)	6 (46.2)	
I	3 (21.4)	2 (15.4)	0.739
II	4 (28.6)	5 (38.5)	
RVSP (mmHg)	44.00 $\pm$ 6.48	39.75 $\pm$ 9.00	0.582

Note: Results are presented as mean  $\pm$  standard deviation (SD) or as absolute values (percentages).

LVEDD – left ventricular end-diastolic diameter; LVESD – left ventricular end-systolic diameter; LA – left atrial diameter; LVEF – left ventricular ejection fraction; TR – tricuspid regurgitation; RVSP – right ventricular systolic pressure.

**Table 3**  
Changes in echocardiographic data after AVR in the improved functional mitral regurgitation (FMR) group

Variables	Preoperative	Late follow-up	<i>p</i> value
Peak gradient (mmHg), mean $\pm$ SD	109.08 $\pm$ 29.07	27.48 $\pm$ 7.25	0.001
Mean gradient (mmHg), mean $\pm$ SD	70.72 $\pm$ 25.44	14.60 $\pm$ 4.6	0.001
LVEDD (mm), mean $\pm$ SD	52.40 $\pm$ 5.92	50.00 $\pm$ 3.38	0.014
LVESD (mm), mean $\pm$ SD	35.20 $\pm$ 7.19	32.08 $\pm$ 5.11	0.006
Septum thickness (mm), mean $\pm$ SD	11.96 $\pm$ 1.88	10.71 $\pm$ 1.40	0.001
Posterior wall thickness (mm), mean $\pm$ SD	11.67 $\pm$ 1.47	10.75 $\pm$ 1.07	0.003
LA (mm), mean $\pm$ SD	43.00 $\pm$ 4.55	41.68 $\pm$ 4.00	0.063
LVEF (%), mean $\pm$ SD	53.60 $\pm$ 14.76	57.40 $\pm$ 7.79	0.140
TR grade, n (%)			
0	14 (56)	12 (48)	
I	5 (20)	8 (32)	0.714
II	6 (24)	4 (16)	
III	0	1 (4)	
RVSP (mmHg), mean $\pm$ SD	44.00 $\pm$ 12.02	37.38 $\pm$ 3.89	0.123

Note: Results are presented as mean  $\pm$  standard deviation (SD) or as absolute values (percentages).

LVEDD – left ventricular end-diastolic diameter; LVESD – left ventricular end-systolic diameter; LA – left atrial diameter; LVEF – left ventricular ejection fraction; TR – tricuspid regurgitation; RVSP – right ventricular systolic pressure.

**Table 4****Comparison of the baseline patient characteristics in respect to EOAI**

Variables	Non PPM (n = 32)	PPM (n = 7)	p value
Age (years), mean ± SD	60.50 ± 10.68	71.00 ± 6.16	0.017
Female sex, n (%)	18 (56.3%)	5 (71.4%)	0.678
BSA (m <sup>2</sup> ), mean ± SD	1.82 ± 0.18	1.85 ± 1.00	0.664
BMI (kg/m <sup>2</sup> ), mean ± SD	25.89 ± 4.97	26.98 ± 3.67	0.588
Atrial fibrillation, n (%)	4 (12.5%)	2 (28.6%)	0.290
NYHA functional class, n (%)			
II	31 (96.9%)	6 (85.7%)	0.331
III	1 (3.1%)	1 (14.3%)	
TAV, n (%)	28 (87.5%)	7 (100%)	1.000
AVA (cm <sup>2</sup> ), mean ± SD	0.62 ± 0.15	0.73 ± 0.27	0.350
Peak gradient (mmHg), mean ± SD	105.16 ± 32.53	90.00 ± 22.95	0.251
Mean gradient (mmHg), mean ± SD	66.19 ± 25.84	59.71 ± 18.98	0.536
LVEDD (mm), mean ± SD	54.41 ± 9.31	54.14 ± 4.53	0.943
LVEDS (mm), mean ± SD	37.63 ± 10.51	36.71 ± 5.62	0.826
Septum thickness (mm), mean ± SD	11.97 ± 1.88	12.00 ± 1.73	0.968
Posterior wall thickness (mm), mean ± SD	11.72 ± 1.57	12.00 ± 1.00	0.654
LA (mm), mean ± SD	42.56 ± 4.98	42.71 ± 2.50	0.938
LVEF (%), mean ± SD	49.69 ± 17.73	47.86 ± 12.20	0.797
TR grade, n (%)			
0	16 (50)	5 (71.4)	0.255
I	6 (18.8)	2 (28.6)	
II	10 (31.3)	0 (0)	
MR grade, n (%)			
2+	23 (71.9)	6 (85.7)	0.653
3+	9 (28.1)	1 (14.3)	

**Note:** Results are presented as mean ± standard deviation (SD) or as absolute values (percentages).

EOAi – effective orifice area index; BSA – body surface area; BMI – body mass index; TAV – tricuspid aortic valve; AVA – aortic valve area; LVEDD – left ventricular end-diastolic diameter; LVEDS – left ventricular end-systolic diameter; LA – left atrial diameter; LVEF – left ventricular ejection fraction; TR – tricuspid regurgitation; MR – mitral regurgitation.

**Table 5****Changes in echocardiographic data after aortic valve replacement (AVR) (EOAi ≤ 0.85 group)**

Variables	Preoperative	Late follow-up	p value
Peak gradient (mmHg), mean ± SD	90.00 ± 22.95	31.86 ± 10.61	0.002
Mean gradient (mmHg), mean ± SD	59.71 ± 18.98	17.14 ± 6.52	0.002
LVEDD (mm), mean ± SD	53.00 ± 3.69	52.17 ± 4.62	0.419
LVEDS (mm), mean ± SD	35.50 ± 5.01	35.83 ± 5.19	0.721
Septum thickness (mm), mean ± SD	11.80 ± 1.79	11.00 ± 1.41	0.374
Posterior wall thickness (mm), mean ± SD	12.00 ± 1.00	11.60 ± 0.89	0.374
LA (mm), mean ± SD	42.67 ± 2.73	42.83 ± 1.72	0.867
LVEF (%), mean ± SD	49.17 ± 12.81	52.50 ± 10.84	0.102
TR grade, n (%)			
0	5 (71.4)	3 (50)	0.157
I	2 (28.6)	2 (33.3)	
II	0 (0)	1 (16.7)	
MR grade, n (%)			
≥ 2+	7 (100)	3 (57.1)	0.125
< 2+	0 (0)	4 (42.9)	

**Note:** Results are presented as mean ± standard deviation (SD) or as absolute values (percentages).

EOAi – Effective orifice area index; LVEDD – left ventricular end-diastolic diameter; LVEDS – left ventricular end-systolic diameter; LA – left atrial diameter; LA – left atrial diameter; TR – tricuspid regurgitation; MR – mitral regurgitation.

*PPM group – before versus after AVR*

Following aortic valve replacement, peak and mean transvalvular pressure gradients reduced significantly. Gradients measured across aortic prostheses were significantly lower than gradients measured across severely stenosed native valve. The remaining echocardiographic parameters did not changed significantly. MR grade

remained ≥ 2+ in 57.1% of patients, and reduced below 2+ in 42.9% ( $p = 0.125$ ) (Table 5).

*Non PPM group – before versus after AVR*

Following aortic valve replacement, peak and mean transvalvular pressure gradients reduced significantly. Gradients measured across aortic prostheses were significantly

lower than gradients measured across severely stenosed native valve. In addition, LVEDD, LVESD, septum thickness, left ventricular posterior wall thickness, also reduced significantly. In other words, there was a significant reverse

remodeling of the left ventricle in this group of patients. Another important finding was that MR grade reduced below 2+ in the majority of patients (65.6%) ( $p = 0.001$ ) (Table 6).

Table 6

#### Changes in echocardiographic data after aortic valve replacement (AVR) (EOAi > 0.85 group)

Variables	Preoperative	Late follow-up	<i>p</i> value
Peak gradient (mmHg), mean ± SD	105.16 ± 32.53	27.25 ± 6.92	0.001
Mean gradient (m, mean ± SD mHg), mean ± SD	66.19 ± 25.84	14.63 ± 4.32	0.001
LVEDD (mm), mean ± SD	54.41 ± 9.31	52.72 ± 8.70	0.030
LVESD (mm), mean ± SD	37.63 ± 10.51	34.97 ± 10.58	0.005
Septum thickness (mm), mean ± SD	11.97 ± 1.88	10.97 ± 1.68	0.001
Posterior wall thickness (mm), mean ± SD	11.72 ± 1.57	10.91 ± 1.49	0.001
LA (mm), mean ± SD	42.56 ± 4.98	41.66 ± 4.50	0.113
LVEF (%), mean ± SD	49.69 ± 17.73	51.91 ± 13.88	0.281
TR grade, n (%)			
0	16 (50)	15 (46.9)	
I	6 (18.8)	8 (25)	
II	10 (31.3)	8 (25)	0.906
III	0 (0)	1 (3.1)	
MR grade, n (%)			
≥ 2+	32 (100)	11 (34.4)	
< 2+	0 (0)	21 (65.6)	0.001

**Note:** Results are presented as mean ± standard deviation (SD) or as absolute values (percentages).

EOAi – effective orifice area index; LVEDD – left ventricular end-diastolic diameter; LVESD – left ventricular end-systolic diameter; LA – left atrial diameter; LVEF – left ventricular ejection fraction; TR – tricuspid regurgitation; MR – mitral regurgitation.

### Discussion

AVR for severe AS decreases left ventricular afterload and initiate reverse remodeling of the left ventricle. Those effects are expected to have positive influence on the mitral valve mechanics, abolishing secondary MR dysfunction without structural abnormalities of the mitral apparatus. Nevertheless, this evolution is frequently not achieved.

Barreiro et al.<sup>8</sup> observed that 82% of patients with functional MR improved postoperatively. Vanden Eynden et al.<sup>9</sup> found that isolated ischaemic and functional MR were the only preoperative factors predictive of MR improvement after AVR. In our study, 64% (25/39) of patients improved FMR postoperatively.

Harling et al.<sup>11</sup> quantitatively demonstrated that, within their review, the structural remodeling resulting from severe AS regresses following AVR, as demonstrated by a reduction in LV mass and LVED diameter. Several studies identified factors associated with evidence of ventricular remodeling, such as higher preoperative LV mass, larger LV diastolic diameter and enddiastolic volume being independent predictors of improvement in MR following AVR. They suggest that, where there is potential for reverse remodeling to occur, a more significant improvement in MR will be seen following AVR<sup>12–14</sup>.

The similar effect was observed in cardiac resynchronization therapy (CRT). Sitges et al.<sup>15</sup> reported that CRT induced acute and sustained reductions in functional MR in almost 50% of patients by initially improving LV systolic

function and dyssynchrony; long-term reverse LV remodeling contributed to this sustained effect.

Our study confirmed the beneficial effect of LV reverse remodeling, with significant reduction in LVEDD, LVESD, septum thickness and left ventricular posterior wall thickness in patients with postoperatively improved FMR.

Numerous studies attempted to identify preoperative patient and echocardiographic characteristics that are predictive of postoperative evolution of MR. Alghamdi et al.<sup>16</sup> in their meta-analysis of 13 non-randomized studies found that progression factors of MR were: LV dysfunction, LA enlargement, atrial fibrillation (AF), peak AV gradient < 60 mmHg, increased LV mass index and increased tricuspid regurgitation (TR) velocity.

Brasch et al.<sup>17</sup> found that elevated left ventricular mass was the only statistically significant predictor of decreased postoperative MR. The only statistically significant predictor of postoperative MR in multivariate analysis, in a study by Joo et al.<sup>18</sup>, was increased right ventricular systolic pressure. Unger et al.<sup>13</sup> published a study indicating that postoperative MR was likely to be improved in patients who had reduced left ventricular function and increased left ventricle size. Jeong et al.<sup>19</sup> demonstrated that patients with preoperative atrial fibrillation and an ejection fraction > 40% were more likely to suffer from residual postoperative MR. Sehovic et al.<sup>20</sup> identified deterioration predictors in patients with moderate-severe FMR: LVEDD > 54 mm, effective regurgitant orifice > 25 mm<sup>2</sup>, regurgitation volume > 40 mL/beat, pulmonary artery systolic pressure > 40 mmHg, LA diameter > 45 mm.

When we compared patients with persistent and improved FMR, the only preoperative echocardiographic and patient characteristics difference was LVEF. Persistent FMR patients had lower preoperative values of LVEF ( $41.79 \pm 17.93\%$  vs.  $53.6 \pm 14.76\%$ ,  $p = 0.033$ ).

The striking findings in our study were postoperative pressure differences between persistent and improved FMR groups. Postoperative peak gradient reduction was 61.4 mmHg ( $t$  test,  $p = 6.881$ ) in the persistent FMR group and 81.6 mmHg ( $t$  test,  $p = 13.282$ ) in the improved FMR group. Postoperative mean gradient reduction was 38.9 mmHg ( $t$  test,  $p = 6.748$ ) in the persistent FMR and 56.1 mmHg ( $t$  test,  $p = 10.653$ ) in the improved FMR group. Obviously, there was a robust reduction in transvalvular gradients in the improved FMR group. This finding could be partially explained by lower preoperative values of LVEF in the persistent FMR group (low flow – low gradient effect).

Previous studies have reported that more than mild PPM, defined as an indexed EOA  $\leq 0.85 \text{ cm}^2/\text{m}^2$ , is associated with less symptomatic improvement, worse hemodynamics at rest and during exercise, less regression of left ventricular hypertrophy, and more cardiac events after AVR<sup>21</sup>. The impact of aortic prosthesis size, and thus of patient/prosthesis mismatch, on the evolution of FMR was addressed in our study. In addition to postoperative peak and mean gradient reduction, LVEDD, LVESD, septum thickness, left ventricular posterior wall thickness, also reduced significantly in patients without PPM. In other words, there was a significant reverse remodeling of the left ventricle in

patients without PPM. Also, FMR grade reduced below 2+ in 65.6% of the non PPM patients, in comparison to 42.9% of the patients in the PPM group. It is worth of mentioning that we used the identical model of mechanical and tissue prostheses in all patients, eliminating the influence of different manufacturer design. In contrary, Waisbren et al.<sup>22</sup> reported that there was no independent relation of aortic prosthesis size with the change in MR.

Our patient selection was guided by restrictive criteria, forming a homogeneous FMR group. Nevertheless, the small number of patients limits the impact of our results, especially in comparing the non PPM and PPM groups (32 vs. 7, respectively).

### Conclusion

In accordance with previous studies, our results also showed improvement in functional MR following AVR surgery, in majority of patients. The reverse remodeling of the LV positively correlates with postoperative FMR downgrading. PPM could be a condition that adversely affects reduction of FMR. We recommend conservative approach in patients with moderate and moderate to severe functional mitral regurgitation, believing that repair or replacement is unnecessary at the time of AVR for severe aortic stenosis. On the other hand, we strongly advocate against PPM in those patients, stressing out the importance of choosing the prosthesis of adequate size.

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## Parenting and externalising problems in adolescents: results of factor analysis

### Roditeljstvo i eksternalizovani problemi adolescenata: rezultati faktorske analize

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#### Abstract

**Background/Aim.** Modern conceptualising of parenting in relation to manifesting adolescence externalising problems is based on parent-child two-way communication, mutual trust and parental involvement. A lot of research reports prove independent contribution of parental variables on expressing externalising problems in middle and late adolescence while data about their mutual relationship are missing. The aim of this study was to determine the relationships of parental variables and variables of externalising problems in a common space. **Methods.** The study included 507 students, both boys and girls, of Belgrade secondary schools, aged from fifteen to eighteen. Parental monitoring was studied using the Parental Monitoring Scale, affective attachment to parents by the Inventory of Parents and Peers Attachment, parenting practices using the Alabama Parenting Questionnaire and externalising problems by the Achenbach System of Empirically Based Assessment, Youth Self-Report. **Results.** Factor analysis indicated the existence of 4 factors (components) which together accounted for 65.5% of variance. The first factor (positive parenting and affective

attachment to mother) accounted for 38.1%, the second (externalising problems and negative parenting) accounted for 11.26%, the third (affective attachment to father) accounted for 8.93% and the fourth (negative discipline and inadequate affective attachment to mother) accounted for 7.22% of variance. The most important discovery arised from matrix correlation was a negative correlation of the second component with the first (-0.539) and the third (-0.481) one. **Conclusion.** A positive relationship between parents and adolescents except for a positive parenting practices of parents characterize all the subscales of parental monitoring except for the parental control. Compared to fathers, mothers are generally more involved in life of adolescents and have better mutual relationship with them, but in relation to externalising problems the affective attachment to fathers proved to be very significant even independently of their parenting role.

#### Key words:

adolescent; parenting; parent-child relations; parents; psychological tests; surveys and questionnaires.

#### Apstrakt

**Uvod/Cilj.** Moderna konceptualizacija roditeljstva u vezi sa ispoljavanjem eksternalizovanih problema u adolescenciji bazirana je na dvosmernoj komunikaciji roditelj-dete, međusobnom poverenju i uključenosti roditelja. Obilje istraživačkih nalaza upućuje na dokaze o nezavisnom doprinosu roditeljskih varijabli na ispoljavanje eksternalizovanih problema u srednjoj i kasnoj adolescenciji, dok nedostaju podaci i njihovom međusobnom odnosu. Cilj rada bio je da se ispita odnos roditeljskih varijabli i varijabli eksternalizovanih problema u zajedničkom prostoru. **Metode.** Ispitivanjem je obuhvaćeno 507 učenika beogradskih srednjih škola uzrasta od petnaest do osamnaest godina, oba pola. Roditeljski

nadzor je ispitivan putem Skale roditeljskog nadzora, afektivna vezanost za roditelje putem Inventara afektivne vezanosti za roditelje, vaspitni postupci roditelja putem Alabama upitnika o roditeljstvu, a eksternalizovani problemi putem Ahenbahovog sistema empirijski bazirane procene. **Rezultati.** Faktori analiza upućuje na postojanje 4 faktora (komponente) koji zajedno objašnjavaju 65,5% varijanse. Prvi faktor (pozitivno roditeljstvo i afektivna vezanost za majku) učestvuje u 38,1%, drugi (eksternalizovani problemi i negativno roditeljstvo) u 11,26%, treći (afektivna vezanost za oca) u 8,93% i četvrti (negativno disciplinovanje i neadekvatno afektivno vezivanje za majku) u 7,22% varijanse. Najznačajniji nalaz proistekao iz matrice korelacije predstavlja negativno korelisanje druge sa prvom (-0,539) i trećom



(0,481) komponentom. **Zaključak.** Pozitivan odnos između roditelja i adolescenata, osim pozitivnih vaspitnih postupaka roditelja, opredeljuju i sve podskale roditeljskog nadzora, sem roditeljske kontrole. Majke su, u odnosu na očeve, generalno uključnije u život adolescenata i ostvaruju bolje odnose sa njima, ali se, u odnosu prema eksternalizovanim

problemima, afektivna vezanost za očeve pokazala veoma značajnom i to nezavisno od njihove vaspitne uloge.

**Ključne reči:**

**adolescenti; roditeljstvo; roditelj-dete odnosi; roditelji; psihološki testovi; ankete i upitnici.**

## Introduction

Studying family and parenting influences on externalising behavioural problems is mostly associated with childhood and early adolescence. Externalising behavioural problems at a later age are usually interpreted as a consequence of some other environmental or biological factors. Research studies that are investigating risk factors of aggressive behaviour and delinquency have recognised following risk factors as prevalent in middle and late adolescence: parental monitoring, poor involvement of parents, rude and inconsistent discipline, rejection by parents and other below<sup>1-4</sup>. Inadequate affective attachment has an impact on externalising problems in middle and late adolescence in combination with other family factors<sup>5,6</sup>.

Current conceptualisation of affective attachment to parents which is based on self-reporting of adolescents and presumes the assessment of mutual trust, communication and alienation has served as a good foundation for researching the attachment with externalising behavioural problems<sup>7-12</sup>. The trust that adolescents have in their parents and belief that they are obliged to communicate to their parents about their personal problems and relationships with their peers seems to be strongly linked with smaller manifestation of externalising problems in adolescence<sup>10</sup>. Adolescents who communicate more about their personal problems and problems with their peers have more trust in their parents. It is indicative that the withholding the private information does not affect the externalising behavioural problems<sup>10</sup>. It can be noticed that there are no differences in correlation between the affective attachment with independent qualities of externalising problems by aggressive behaviour and rule-breaking behaviour<sup>8,13</sup>. Namely, research findings confirmed that adolescents who had lower scores on the subscales of trust and communication and higher scores on subscale of alienation, had higher scores in subsequent measuring both in aggressive behaviour and in rule-breaking behaviour. Furthermore, higher scores on the scales measuring aggressive and rule-breaking behaviour had a feedback effect on the weakening of affective attachment at the third point of measuring<sup>8</sup>. It was determined that affective attachment to parents mediates in explaining the influence of parents' negative control on manifestation of externalising problems<sup>14</sup>. Although the mediator significance of affective attachment is being lost at an older age the relationship of positive and negative parenting variables with affective attachment to parents remains relevant in all age groups<sup>14,15</sup>. In a research conducted on the basis of exploring 218 dyadic relations between mothers and adolescents it was found that the parents' confidence in adolescents can mediate in explaining the influence of child dis-

closure, parental solicitation and different strategies of hiding the information with manifestation of rule-breaking behaviour<sup>16</sup>. The authors confirm correlation of affective attachment to parents and parental monitoring even independently of externalising problems confirming that safe patterns of affective attachment to parents influence a more adequate parents' monitoring and better children and adolescents disclosure<sup>15,17</sup>. Results of the research indicate that affective attachment in the context of delinquency loses its significance with the age in difference to parental monitoring whose relation with delinquency shows stability<sup>18-20</sup>. In regard to this the authors point out a principal idea of the theory of affective attachment meaning that style, that is, quality of affective attachment to parents, mediates between parenting and delinquency<sup>21,22</sup>.

Research findings indicate that there are differences in manifestations of externalising problems in relation to affective attachment of adolescent to parental figures of different gender. Affective attachment to mother and general affective attachment to father and mother proved significant for externalising problems in adolescents<sup>23</sup>. Research studies in western societies also reported that the adolescents are statistically more significantly attached to mother than to father what can be associated with smaller involvement of father in their upbringing<sup>24,25</sup>. Regarding the relationship with externalising problems (according to the reports of the teachers) affective attachment to fathers and involvement of fathers explained the highest percent of variance. Furthermore, over 20% adolescents in an American study do not live with their fathers what can have consequences on their less involvement and quality of affective attachment<sup>24</sup>. On a sample of Spanish adolescents it was established that poor affective attachment to fathers influences manifestation of aggressive behaviour in adolescents of both gender<sup>15</sup>. On the other hand, affective attachment to mother was connected with externalising problems according to reports of the adolescents in all age groups<sup>10-19</sup>, while affective attachment to fathers from the age of 12 is gaining significance<sup>14</sup>. According to the reports of adolescents one-way relationships were discovered between externalising problems and affective attachment to fathers on one hand and affective attachment to mothers and adolescents externalising problems on the other<sup>26</sup>.

Previously discussed research reports suggest that in order to understand adolescents externalising problems it seems essential to understand the relationship between variables of parental monitoring, affective attachment to parents and parenting practices. By discussing studied constructs in common space we presumably attain a more complete and wider picture of the relationship of studied parental variables and externalising problems. It can, therefore, serve as a basis

for further directives in modelling the effects that parental variables can have on externalising problems. Moreover, for affective attachment the gender of parent is important, more than parental monitoring or parenting practices in the context of relationship with externalising problems.

The aim of this study was to determine the mutual correlation between parental variables and variables of externalising problems in common space.

## Methods

The research was conducted during the second term of the 2013/14 school year. Research participants were the pupils of six Belgrade secondary schools and they were chosen randomly from one class in each grade (from the first to the fourth grade). The research included three secondary schools and three specialised vocational schools. The sample included 507 pupils of both gender aged from fifteen to eighteen. Pupils' average age was 16.69 (standard deviation = 1.119) years. The sample was equalised according to age, gender and type of school. Data were collected from the pupils by a questionnaire. Questionnaire completion was anonymous. The time needed for completing the questionnaire lasted as one school lesson. The pupils were previously instructed orally and in written form how to fill in the questionnaire.

### *Instruments*

For the assessment of externalising problems, a part of instrumentarium for assessing the emotional, social and behavioural problems of children and adolescents, the Achenbach System of Empirically Based Assessment, Youth Self-Report<sup>27</sup> was used. The version of questionnaire used was standardised to suit the children aged between 11 and 18 years. Prevalence and manifestation forms of externalising problems were studied on the basis of the Scale of Aggressive Behavior and the Scale of Rule-Breaking Behavior. The scales are three grade Likert-type scale where respondents give their answers by choosing one of the replies offered starting from 0 – false, through 1 – partly true, and 3 – true. Total score of externalising problems and the scores on the Scale of Aggressive Behaviour and the Scale of Rule-Breaking Behaviour are being obtained by the addition of answers, higher scores implying larger share of externalising behaviour problems. The coefficients of Cronbach's alpha for the Scale of Aggressive Behaviour (0.837) and for the Scale of Rule-Breaking Behaviour (0.824) reflect a good internal consistency and correspond to the findings of other researchers<sup>27</sup>.

The Scale of Parental Monitoring<sup>28, 29</sup> was used for the assessment of parental monitoring. The scale of parental monitoring consists of four sub-scales titled: Parents' Knowledge, Child Disclosure, Parental Solicitation and Parental Control. The coefficients of reliability, according to the results obtained by studying the Sub-scales of Parental Monitoring, are mainly deemed to be good or acceptable, the coefficients of reliability for Subscale of Parents' Knowledge be-

ing 0.849, Subscale of Parental Control being 0.832, Subscale of Child Disclosure being 0.746, with the exception of Subscale of Parental Solicitation whose reliability is, taking into account the obtained value of 0.676, considered questionable. Compared to the results obtained by some other authors similar values of Cronbach's alpha<sup>29</sup> were established.

The Inventory of Parents and Peer Attachment<sup>30, 31</sup>, the section for assessing the affective attachment to mother and father according to the last amendments made by the author<sup>31</sup>, was used for assessing the affective attachment. The instrument measures the adolescents' perception of positive and negative affective and cognitive dimension of the relationship with parents and close friends – particularly how well they can serve as sources of psychological safety. It was anticipated for ages from twelve to twenty. It contains twenty-five items grouped in three subscales titled: Trust, Communication and Alienation. In conformity with the findings of other authors, a revised version of the Inventory of Parents Affective Attachment used in this research paper, except for the Subscale of Alienation from Mother, has good reliability parameters<sup>32</sup>.

The Alabama Parenting Questionnaire (APQ) measures five parenting dimensions that are relevant for aetiology and treatment of externalising problems, the version intended for six to 18-year old children<sup>33</sup>. It contains 42 items grouped in five subscales in a following way: Parental Involvement, Positive Parenting, Poor Monitoring/Supervision, Inconsistent Discipline and Corporal Punishment. The Alabama Parenting Questionnaire<sup>34</sup> has poorer internal consistency as was being reported by some other authors, although within acceptable limits. For the subscales: Positive Parenting of 0.809 and Parental Involvement of 0.792 the value of Cronbach's alpha indicates good, that is, acceptable reliability. However, the coefficients measured for subscales: Corporal Punishment 0.662 and Poor Monitoring/Supervision 0.688, indicate suspicious reliability taken that subscale of Inconsistent Discipline has a poor reliability (0.523).

The scales of parental monitoring, affective attachment to parents and parenting questionnaire are five-grade scales of Likert-type scale, in which the pupils reply by choosing one of the answers offered from 1 – never, 2 – rarely, 3 – occasionally, 4 – often to 5 – almost always. The subscales scores are obtained by addition of the answers, the higher scores on subscales implying a higher share of aforementioned aspects.

### *Statistical analysis*

Data were processed by a SPSS statistical programme package. Instruments reliability was examined by means of Spearman-Brown-Kuder-Richardson-Guttman (Cronbach alfa) test. Exploratory data analysis of major components was carried out by the measures standardised by means of Bloom's algorithm. It was applied in order that in mutual space of all examined variables the factors with maximum share in total variance of original variables should be extracted. A parallel analysis was used for determining the number of isolated components.

## Results

Factor analysis of applied instruments was carried out by means of the analysis of the principal components in mutual space of all variables. Random characteristic values with number of variables and a sample size were both generated as parameters by parallel analysis. Randomly generated values were compared to actual ones obtained in the research. The factors with higher internal values than ran-

domly generated ones were retained. Hence, four components whose characteristic values exceed 1 were extracted. Total percent of variance explained by these components was 65.54%, the contribution of the first component being the highest 38.11%, second 11.8%, third 8.93% and fourth 7.24%. The extracted components were then rotated by help of Promax algorithm with Kaiser normalisation ( $\kappa = 4$ ). Table 1 shows the matrix of the structure of extracted components.

**Table 1**

**Matrix of the structure of extracted components (1, 2, 3, 4)**

Variables	Extracted components (randomly generated characteristic values)			
	1 (1.328)	2 (1.264)	3 (1.212)	4 (1.170)
Empirical characteristic values	6.478	1.916	1.519	1.231
Parental involvement	0.987			
Communication mother	0.876			
Parental solicitation	0.735			
Trust mother	0.706			-0.354
Child disclosure	0.671			
Positive parenting	0.660	0.315		
Parental knowledge	0.533	-0.325		
Alienation mother	-0.522			0.371
Agressiveness		0.813		
Rule-breaking		0.804		
Poor monitoring/supervision		0.709		-0.448
Inconsistent discipline		0.676		
Trust father			0.903	
Communication father			0.899	
Alienation father			-0.836	
Parental control				0.807
Corporal punishment				0.511
Percent of explained variance	38.11	11.26	8.93	7.24
Total percent of explained variance		65.54		

**Extracted components: 1 – positive parenting and affective attachment to mother ; 2 – externalising problems and negative parenting; 3 – affective attachment to father; 4 – negative discipline and inadequate affective attachment to mother.**

The first isolated component (positive parenting and affective attachment to mother) integrates subscales of the Alabama Parenting Questionnaire indicating positive parental practices (saturation being 0.987 for the the Subscale of Parental Involvement and 0.660 for the Subscale of Positive Parenting); all the subscales of the Scale of Parental Monitoring (saturation being 0.735 for the Subscale of Parental Solicitation, 0.671 for the Subscale of Child Disclosure and 0.533 for the Subscale of Parental Knowledge), except for the Subscale of Parental Control; and all the subscales of the Inventory of Parents and Peers Attachment in relation to mother (saturation being 0.876 for the Subscale Communication with Mother, 0.706 for the Subscale of Trust in Mother and -0.522 for the Subscale of Alienation from Mother).

The second component (externalising problems and negative parenting) was dominantly determined by the scales of externalising problems with saturations being 0.813 for the Scale of Aggressive Behaviour and 0.804 for the Scale of Rule-breaking Behaviour, subscales of the Alabama Parenting Questionnaire indicating negative parenting practices

with saturations being 0.709 for the Subscale of Poor Monitoring/supervision and 0.676 for the Subscale of Inconsistent Discipline, and negative saturation of the Subscale of Parental Knowledge -0.325 and positive saturation of the Subscale of Positive Parenting of 0.315.

The third component (affective attachment to father) was constituted by all the subscales of the Inventory of Parents and Peers Attachment regarding father (saturation being 0.903 for the Subscale of Trust in Father, 0.899 for the Subscale of Communication with Father and -0.836 for the Subscale of the Alienation from Father).

The fourth component (negative discipline and inadequate affective attachment to mother) was saturated by following subscales: Parental Control (0.807), Corporal Punishment (0.511), Poor Monitoring/supervision (-0.448); Alienation from Mother (0.371), and Trust in Mother (-0.354).

The most significant finding which issued from the matrix of correlation was a negative correlation of the second component with the first (-0.539) and third (-0.481) one (Table 2).

**Table 2**  
**Matrix of correlation of extracted components**

Extracted components*	1	2	3
1			
2	-0.539		
3	0.481	-0.481	
4	0.009	0.074	-0.108

\*For explanation see under Table 1.

## Discussion

Isolating the constructs that indicate a positive relationship with parents and affective attachment to mother as the first major components represents a significant finding. Positive aspects of relationship with parents including the parents' interest, presence and knowledge about where, who with and how their children spend their free time indicate specificity of the nature of parenting as an active, supporting and two-way process<sup>35, 36</sup>. The results of the research indicate that mothers are generally more involved in the life of adolescents, they accomplish better communication, adolescents have greater confidence in them and self-report to them more about where, with whom and how they spend their time without parental monitoring<sup>10, 37, 38</sup>. Research results support the thesis on differences in affective attachment to father and mother, except in relation to variables of parenting and parental monitoring and manifestation of externalising problems<sup>14, 15</sup>.

Aggressive behaviour and rule-breaking behaviour dominantly saturate second component in such a way that poor monitoring and inconsistent discipline have high positive saturation, what conforms to the findings of other studies on structuring externalising problems in behaviour and main negative aspects of parenting associated with it<sup>19, 34, 39</sup>. Negative saturation of parents' knowledge about children's whereabouts, friends and activities corresponds to the assumptions about the importance of this construct in relation to aggressive behaviour and rule-breaking behaviour, and even greater importance in relation to negative parental practices, like poor monitoring/supervision and inconsistent discipline<sup>4, 40, 41</sup>. However, what surprised us was a positive saturation of positive parenting about which rare empirical evidences can be found<sup>42, 43</sup>. It could be explained by so called reactive parenting when parents after having perceived the problems in adolescents' behaviour are trying to compensate for the lost time<sup>42, 44</sup>.

Bearing in mind that there are only few studies that research the affective attachment to father in the context of perceiving the relationships with parents and externalising problems, the research findings that the third component is

saturated by trust, communication and alienation from father are thought very significant. The results of sporadically conducted studies indicate utterly different paths in adjustment, origin and maintenance of the problems in behaviour, especially externalising problems in adolescents in whom there are differences regarding variables of relationship to father<sup>15, 24, 45</sup>. Namely, research findings indicate that affective attachment to fathers in relation to expressing externalising problems becomes particularly apparent after the age of 12<sup>14</sup>.

The constructs which saturate fourth component reflect parental practices which indicate strict discipline manifested by corporal punishment, parental control, strict monitoring and supervision with lack of trust and with alienation from mother, what corresponds to the findings of other authors<sup>46-49</sup>. Variables of affective attachment to mother often mediate in explaining the externalising problems by negative parental variables, the link between parental practices and affective attachment being more stable than that which is being realised with externalising problems<sup>14, 15</sup>. The authors dispute about presigns and strength of variables of parental control and relationship with externalising problems in connection thereof<sup>29, 37</sup>. Taking into account that parental control is manifested by limiting freedom by setting rules and restrictions on where the adolescents are allowed to go, how long they are allowed to stay, what activities they can do and so on<sup>28, 29</sup> it can be said that it is about the construct that represents a negative aspect of parental monitoring and discipline what was confirmed in this research as well.

## Conclusion

A positive relationship between parents and adolescents, except for positive parental practices, determine all subscales of parental monitoring too, except parental control. Mothers, compared to fathers, generally realise better relationship with adolescents, they are more involved in the life of adolescents, offer them more support so that the adolescents disclose their activities to mothers more often. On the other hand, affective attachment to father has an important influence on adolescents externalising problems in contrast to mothers and independantly of their parenting role.

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## Social support in recovery former users of psychoactive substances in Montenegro: A survey study

### Socijalna podrška u periodu oporavka bivšim korisnicima psihoaktivnih supstanci u Crnoj Gori: Istraživačka studija

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#### Abstract

**Background/Aim.** Social support can be a very powerful and beneficial force in the recovery process. Research of social support as an important component in the process of resocialization of former users of psychoactive substances, so far, has been neglected in Montenegro. However, one of the conditions for quality analysis whose outcome would involve examining of deeper causal relationships is examining of social support structure of the respondents. That is why the main goal of this study was to determine precisely dimension of social support, and its factorability. A special sub-aim was to identify latent structure of emotional support as specific dimension within the social support scale. **Methods.** The survey was conducted with 107 clients treated in the Public Institution for Accommodation, Rehabilitation and Resocialization of Users of Psychoactive Substances in Podgorica (Montenegro) from May 2014 to October 2016. The Multidimensional Social Support Scale (MSPSS) was used. It consists of 12 variables that measure three components of support: Family, Friends and Significant Others. The analysis of the main components with

direct oblimin rotation was used to examine the factorability of the MSPSS. After factor analysis conducted, the reliability of the determined scale was tested by Cronbach alpha coefficient through discriminatory validity. **Results.** All three components showed statistically significant results ( $p < 0.05$ ). The coefficient of correlation between Friends and Significant Other was 0.510, while between Significant Others and Family it scored 0.617. On the other hand, the coefficient of correlation between Significant Other and Family was 0.525. As we had assumed and as previous results in this area suggested there was a significant link between the Friends and Family components. Also, 85.1% of the respondents stated that social support is important (or extremely important) in the process of rehabilitation and resocialization. **Conclusion.** Survey emphasizes the important role of the family in the life of respondents. Social support has many benefits and it is often crucial to establishing successful recovery of former users of psychoactive substances.

**Key words:** substance-related disorders; therapeutics; social support; family.

#### Apstrakt

**Uvod/Cilj.** Socijalna podrška ima veoma značajnu ulogu u procesu oporavka bivših zavisnika od psihoaktivnih supstanci. Istraživanje socijalne podrške kao važne komponente u procesu resocijalizacije bivših korisnika psihoaktivnih supstanci, do sada je bilo zanemareno u Crnoj Gori. Međutim, jedan od uslova za kvalitetnu analizu, čiji ishod uključuje ispitivanje dubljih uzročnih odnosa, jeste ispitivanje strukture socijalne podrške ispitanicima. Zbog toga je glavni cilj ovog rada bio da precizno odredi dimenziju socijalne podrške i njenu faktorsku vrednost. Poseban cilj bio je identifikacija latentne strukture emocionalne podrške kao specifične dimenzije unutar skale socijalne podrške. **Metode.** Istraživanje je uključilo 107 zavisnika lečenih u Javnoj ustanovi za smeštaj, rehabilitaciju i

resocijalizaciju korisnika psihoaktivnih supstanci u Podgorici (Crna Gora) u periodu od maja 2014. do 1. oktobra 2016. godine. U istraživanju je korišćena Multidimenzionalna skala socijalne podrške (MSPSS) koja se sastoji od 12 varijabli koje mere tri komponente podrške: porodice, prijatelja i značajnih drugih. Analiza glavnih komponenti sa direktnom *oblimin* rotacijom (*direct oblimin rotation*) korišćena je za ispitivanje faktora bilnosti MSPSS. Nakon sprovedene faktorske analize, pouzdanost skale je testirana pomoću Cronbach-ovog koeficijenta alfa (*Cronbach alpha coefficient*) kroz diskriminatornu validnost. **Rezultati.** Sve tri komponente pokazale su statistički značajne razlike ( $p < 0,05$ ). Koeficijent korelacije između komponenti Prijatelji i Značajni drugi iznosio je 0,510, dok je između Značajnih drugih i Porodice iznosio 0,617. S druge strane, koeficijent korelacije između Značajnih drugih i Porodice bio je

0,525, što ukazuje da postoji značajna veza između komponenti Prijatelja i Porodice. Takođe, 85,1% ispitanika navelo je da im je socijalna podrška važna (ili izuzetno važna) u procesu rehabilitacije i resocijalizacije. **Zaključak.** Istraživanje naglašava važnu ulogu porodice u životu ispitanika. Socijalna podrška ima

puno prednosti i često je od ključnog značaja za uspešni oporavak bivših korisnika psihoaktivnih supstanci.

**Ključne reči:**  
zavisnost od supstanci; lečenje; socijalna podrška; porodica.

## Introduction

Dependence on psychoactive substances is considered to be physical, mental, social and spiritual illness<sup>1</sup>. In the last 40 years, a growing trend of number of addicts has been recorded worldwide<sup>2</sup>. Relapse is one of the most important topics in the recovery period<sup>3</sup>. It is therefore of utmost importance to determine which factors influence on the prevention of relapse.

Social support is determinant of addiction and due to its multidimensionality it can be defined from different perspectives and operationalized in different ways<sup>4</sup>. The sources of social support are numerous and quite diverse, including family, friends, partners, community and associates<sup>5</sup>.

Cohen and Wills<sup>6</sup> mention several types of social support. Informational support is important for understanding and dealing with problems, and in literature it is also called counseling and assessment support. Self-esteem support is a person's information that he/she is accepted and respected. By communicating with people who accept and respect him/her, regardless of his or her difficulties or failures, a person develops self-esteem and this type of support is called emotional or close support. The need of each individual for belongingness is met through social companionship in leisure activities, while instrumental support is the one that provides for material support. Empirical research shows high correlations of various social support functions<sup>6</sup>. Family is a dominant source of sociability and social support<sup>7-10</sup>. Close relatives are more often a source of emotional and instrumental support, while friends are more important for socializing<sup>11-12</sup>. Instrumental support is often provided by neighbors<sup>13, 10</sup>. Social relationships are assessed by frequency of social interactions<sup>14</sup> and analyzed through three spheres. The primary sphere implies the closest family relationships; secondary relates to friends, relatives and a closer social community while tertiary relates to participation in organized activities and associations<sup>15</sup>. Böhnke<sup>16</sup> warns of the importance of family cohesiveness, intergenerational solidarity and friendships. In their study, Spoth and Redmond<sup>17</sup> dealt with the role of social support in the period of treatment and prevention of relapse. The authors suggest that the existence of supporting structures and networks plays a significant role during the drug treatment process in people who abuse drugs and in preventing relapse while contributing to the improvement of mental health.

Although the problem of the use of psychoactive substances is a widespread phenomenon, post-rehabilitation and resocialization social support did not find its place in scientific research in Montenegro. This problem can be also seen as a global one. Every adequately conscious society should be interested in providing social support to clients after rehabilitation and resocialization.

The aim of the research of social support as an important component in the process of resocialization of former users of psychoactive substances, so far, has been neglected in Montenegro. However, one of the conditions for quality analysis whose outcome would involve examining of deeper causal relationships is examining of social support structure of the respondents. That is why the main goal of the study was to determine precisely dimension of social support and its factorability. A special sub-aim was to identify latent structure of emotional support as specific dimension within the social support scale.

## Methods

The survey covered 107 clients, former users of psychoactive substances and former residents of the Public Institution for Accommodation, Rehabilitation and Resocialization of Users of Psychoactive Substances in Podgorica, Montenegro. The sample included respondents who completed one-year rehabilitation and resocialization period. The survey was conducted by face-to-face method with the prior approval of the Institution in which the survey was conducted as well as the voluntary consent of the respondents. The total sample covers 42.8% of the total number of clients of the Institution for the survey period.

The Multidimensional Scale of Perceived Social Support (MSPSS) was used for this survey<sup>18</sup>. The scale consists of 12 items indicating dimensionality (factor validity) expressed through three components: Family, Friends, and Significant Others. Answers in the Likert scale were ranked from 1 to 7 (1, I strongly disagree – 7, I strongly agree). One of the goals was to validate this scale on our sample. By examining the internal compliance of the data, it was found that the results obtained by analysis coincided with the original results of the author of this scale.

The analysis of the main components with direct oblimin rotation<sup>19</sup> was used to examine the factorability of the MSPSS. After factor analysis conducted, the reliability of the determined scale was tested by Cronbach alpha coefficient through discriminatory validity.

## Results

Some of the key sociodemographic characteristics of respondents implied that majority of them (70.1%) completed secondary school, their average income amount was to 720 Euros (distribution of data indicates asymmetry and presence of below-average values; skewness = 1.598). The largest number of them was raised in a complete family (81.3%) (Table 1).



**Table 1**  
**Sociodemographic characteristics of respondents**

Variables	n (%)	Skewness	Kurtosis
Education (multiply responses)			
elementary school	21 (19.6)		
secondary school	75 (70.1)		
faculty	11 (10.3)		
Type of family (multiply responses)			
complete	87 (81.3)		
single parents	9 (8.4)		
expanded family	8 (7.5)		
other	3 (2.8)		
Marriage status of parents			
married	54 (50.5)		
a marital union	1 (0.9)		
divorced	12 (11.2)		
one parent died	36 (33.6)		
missing values	4 (3.7)		
Total family members, mean $\pm$ SD	4.09 $\pm$ 1.24	0.028	-0.521
Income (Euros), mean $\pm$ SD	720.70 $\pm$ 461.38	1.598	2.960

**Table 2**  
**Relationship with parents**

Relationship with parents	Respondents (%)	
	with mother	with father
Very close	36.4	20.6
Close	40.2	30.8
Neither close nor distant	17.8	24.3
Distanced	4.7	12.1
Very distanced	0.9	10.3

**Table 3**  
**Relationship with partners**

Relationship with partners	Respondents, n (%)
Very close	23 (21.5)
Close	13 (12.1)
Neither close nor distant	5 (4.7)
Distanced	5 (4.7)
Very distanced	5 (4.7)

Most of the clients stated that they had close and very close relationships with mother and father, and the smallest percentage were at a great distance with mother and father.

**Table 4**  
**Factor weight for principal component analysis (PCA) with direct oblimin rotation (Kaiser normalization) of the three-component solution**

Items	Factor weights		
	Friends	Significant Other	Family
I can count on my friends when things go wrong	0.944	-0.002	-0.018
I have friends with whom I can share my happiness and sorrow	0.923	-0.017	-0.002
I can talk about my problems with my friends	0.856	-0.087	0.065
My friends really try to help me	0.764	0.160	-0.003
There is a special person who is there always when I am in need	-0.072	0.941	0.066
There is a special person who is a source of comfort to me	0.075	0.865	0.027
There is a special person in my life with whom I can share joy and sorrow	-0.017	0.761	-0.034
There is a special person in my life who cares about my feelings	0.278	0.417	0.095
I can talk about my problems with my family	-0.021	0.064	0.855
My family is willing to help me make decisions	-0.003	-0.001	0.822
I have the emotional help and support I need from my family	-0.014	-0.078	0.780
Kaiser-Meyer-Olkin's indicator	0.092	0.119	0.727
Bartlett's sphericity test			0.845
			0.000

Approximately one third of the respondents (33.65%) described their relationship with partners as very close or close, while 9.4% of the respondents had a distant and mostly sympathetic relationship with their partners (Tables 2 and 3).

#### *Results of the main components analysis*

Analysis of the main components separated the components and determined factorability within the three components. Prior to the analysis of the main components, the adequacy of the data was determined by examining the correlation between the variables ( $r > 0.3$ ). The value of Kaiser-Meyer-Olkin's index was 0.845 which exceeded the threshold of 0.6 with statistically significant Bartlett's test of sphericity ( $p = 0.000$ ).

The analysis of the main components revealed the presence of three components with characteristic values above 1, which accounted for 54.89%, 12.53% and 10.92% of variance. With regard to the structure of the components, these were entitled Friends, Family, and Significant Others, respectively. Looking at Table 4, one can notice the structure of the components.

For example, the Friends component explains the variables that indicate friends as an important support and encouragement in life. On the other hand, the Significant Others component includes variables that imply the existence of a “special person” in the life of respondents and his/her significant role. In the Family component the presence of variables that emphasize the important role of the family in the life of respondents was also observed.

#### *Internal compliance of the scale*

In order to examine the internal compliance of the scale, we examined the Cronbach alpha coefficient, which examined both for the entire score of variables that make up the synthetic variable – social support as well as for the individual synthetic variables extracted in the previous analysis. Zimet et al.<sup>18</sup> had previously tested these properties and determined the following: the coefficient  $\alpha$  for all 12 variables (Social support) was 0.88. The Family, Friends and Significant Others showed coefficient  $\alpha$  of 0.85, 0.75, and 0.72, respectively. In comparison, our analysis established internal compliance of the Social Support of 0.92, while for the Family, Friends and Significant Others these coefficients amounted to 0.89, 0.934, and 0.88, respectively.

#### *Emotional support within the MSPSS scale*

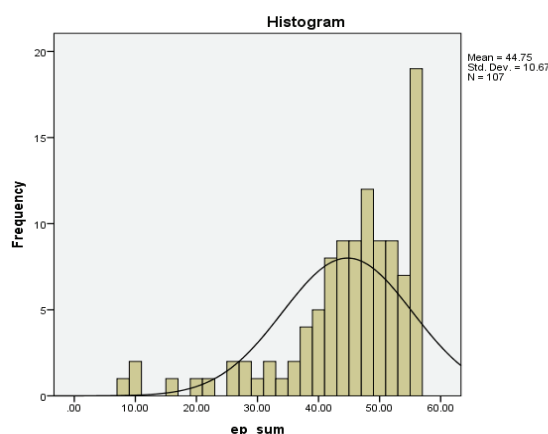
Emotional support as a sum of the two most common components, in our case the Friends and Family, is an important if not the most important part of social support. By examining the correlation coefficients among the three components of the MSPSS, the validity of this assumption was determined. All three components showed statistically significant results ( $p < 0.05$ ). The correlation coefficient between the Friends and Significant Others was 0.510, while between the Significant Others and Family it scored 0.617. On the other hand, the correlation coefficient between the Significant Others and Family was 0.525. As we had assumed and as previous results in this area suggested there was a significant link between the Friends and Family components. Due to the lack of strong statistical evidence, these data represent a sufficient indicator of the accuracy of the assumptions stated in the paper.

In order to examine significance of emotional support, it was formatted synthetic sketch of variables identified in the previous section as a part of emotional support. As Table 5 shows, emotional support for former users of psychoactive substances had a big importance. The distribution values ranged from 8 to 56. The arithmetic mean was 44.75 and the value of the skewness had negative asymmetry.

**Table 5**

**Descriptive statistics of Emotional Support**

n	107	107
	0	0
The arithmetic mean		44.7477
Standard error		1.0315
Median		47.0000
Modus		56.00
Std. Deviation		10.67009
Variance		113.851
Skew		-1.527
Kurtosis		2.494



Data from Table 6 (after the interval variables separation and transformation into categorical ones) showed the importance of emotional support to the respondents. Very small percentage of respondents expressed that emotional support was not important, while 85.1% of them pointed out the importance of this construct.

**Table 6**

**Emotional support**

Emotional support	Respondents n (%)
It does not matter to me at all	4 (3.7)
It is a bit important to me	4 (3.7)
It is neither important nor irrelevant to me	8 (7.5)
It is important	35 (32.7)
It is extremely important	56 (52.3)

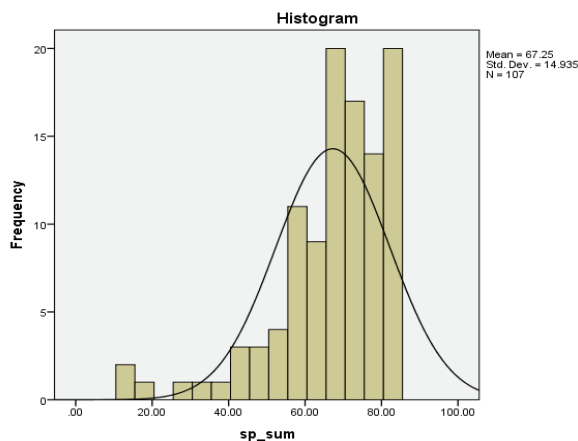
#### *Social support to former users of psychoactive substances*

In order to determine level of social support to former users of psychoactive substances, synthetic sketch of variables, which make this construct, was formed.

Values for variable Social support are given in Table 7. Their range was from 13 to 84. The higher value on the scale implies presence of greater importance of social support. In average values we noticed some disagreement and values of arithmetic mean, medium, media, and mode significantly deviate and point to the asymmetry of the distribution. The sketch indicator, which measures distribution asymmetry, showed us a negative asymmetric distribution. This implies presence of above-average values.

**Table 7****Descriptive statistics of Social Support**

n	Valid	107
	Missing	0
The arithmetic mean		67.2523
Standard error		1.44377
Median		70.0000
Modus		84.00
Std. deviation		14.93451
Variance		223.039
Skew		-1.574
Kurtosis		3.116



Variables were transformed into a material with 5 categories demonstrating an importance of social support to the respondents. Cumulatively, 85.1% of the respondents stated that social support was important (or extremely important) (Table 8).

**Table 8****Social support**

Social support	Respondents n (%)
It does not matter to me at all	3 (2.8)
It is a bit important to me	3 (2.8)
It is neither important nor irrelevant to me	10 (9.3)
It is important	37 (34.6)
It is extremely important	54 (50.5)

**Discussion**

This aim of this paper was to identify the perception of social support of former users of psychoactive substances in Montenegro following their rehabilitation and resocialization. The results showed that the scale used has good internal and test-retest reliabilities and moderate construct validity<sup>18</sup>.

In 2011, a survey in Vietnam showed that parents, wives, brothers and sisters gave most of emotional support to former addicts. Respondents reported that abstinence was maintained thanks to the support of family members, and emotional support by family was emphasized for day to day functioning. Also emotional support provided by families made a significant contribution to tackling obstacles and problems they encountered and often provided strong motivation to abstain from drugs, care for their health and seek employment<sup>20</sup>. Those results are very similar with results from Montenegro where the highest percentage respondents quoted the importance of this construct.

A research carried out in 2015 in China pointed to the importance of social support in recovery period and its significance in long rehabilitation period of addicts<sup>21</sup>. Our research showed that for more than three quarters of respondents social support is important or extremely important. Family support proved to be important for the treatment process, while good relationships with other significant

persons in life are a significant factor for mental health of clients, and are particularly important from the perspective of social functioning<sup>22</sup>. For clients who are in the program for treatment of psychoactive substances abuse, family support may be the most important aspect of social support<sup>18, 23</sup>. Clients who perceive family support as good believe to have a safe environment, adequate health and social protection, financial support, possibility to use social resources<sup>24, 25</sup>, which largely reflects on their quality of life.

The results of our research correlate with the results carried out by Shahzad et al.<sup>26</sup> with clients who underwent treatment for drug addiction in rehabilitation centers in Pakistan. Their research has shown that availability of social support by the family, friends and employees in the treatment facilities helps clients to better cope with the addiction and is a significant factor in preventing relapse.

In similar researches, the authors concluded that social and emotional support has a significant role after period of rehabilitation and resocialization. Therefore we can conclude that results from our survey are reliable and that cultural differences did not affect them.

*Limitations of the study*

The analysis pointed to interesting factorability, but more significant research should involve a larger sample. This is especially important in cases of applying multivariate statistical methods, which is one of the prerequisites for their application. This would be particularly significant for determining correlation between the components. Since all variables were a part of one scale (social support), the question is how much their mutual causality affects correlation between the components.

*Benefits of the study*

Taking into account the pioneering contribution of the study to understanding the current problem, especially considering the inadequate examination of the topic in Montenegro, an opening of this insufficiently explored issue sets the foundation for further research in this field.

## Conclusion

Results of this study suggest that emotional support plays an important role in the perceived social support after the period of rehabilitation and resocialization of former

users of psychoactive substances. It is recommended that social support is promoted through intervention programs in dealing with clients in the process of rehabilitation and resocialization.

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## Principal characteristics of patients acutely poisoned by ethanol in the region of Belgrade (Serbia)

Osnovne karakteristike pacijenata akutno otrovanih etanolom na području Beograda (Srbija)

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### Abstract

**Background/Aim.** Acute intoxication by ethanol constitutes a significant part of the acute pathology caused by toxic chemicals, which require medical care in specialized health care institutions. The aim of the study was to determine some principal characteristics of the patients treated after acute ethanol poisoning, such as: participation in the total number of patients treated after acute poisoning by all chemical agents, age, sex, severity of poisoning and capacities for ambulatory or hospital treatment. **Methods.** A five-year retrospective case study was conducted on 20,891 acute poisoned patients, of which 10,731 were treated after acute ethanol poisoning during the period 2011–2015. All the subjects satisfied the diagnostic criteria of poisoning according to the World Health Organization International Classification of Diseases-10 and standardized Poison Severity Score scale. **Results.** Monitored parameters were analyzed in 10,731 patients acutely poisoned by ethanol, and their average number during one study year was  $2146.2 \pm 437.95$ . The average number of treated patients due to poisoning by ethanol ( $51.47 \pm 2.86\%$ ) was statistically significantly higher than that of other causes of acute poisoning ( $p < 0.001$ ). The majority of poisoned patients were men, aged 19 to 65 years, with mild to moderate symptoms of acute ethanol intoxication. The highest

number of patients poisoned by ethanol had Poison Severity Score 1 ( $70.25 \pm 5.04\%$ ,  $p < 0.001$ ). Very few acute poisoned with ethanol required hospital treatment (1.05%). Anticipated number of patients acutely poisoned by ethanol had high percentage of increase for the period 2016–2020 (slightly more than 20%), which is worrying, primarily due to their high absolute number. **Conclusion.** Acute alcohol poisoning represents a significant part of the pathology in the total number of patients treated due to acute poisoning with various chemical substances. The results of this analysis showed that certain populations are particularly vulnerable to abuse of alcohol to the level requiring health care (male population, age 19–65 years). Only a small number of patients (1% of all patients treated for acute alcohol poisoning) required clinical treatment. The obtained data provided the basis for a more targeted preventive action in certain population groups, as well as the adequate planning of professional medical staff engagement, material, spatial and other capacities. These data, also, provided the basis for additional detailed social, economic, health and other researches in this area as well as a more detailed registration of this type of poisoning and establishing a monitoring system and database.

**Key words:** ethanol; poisoning; serbia; demography; risk factors.

### Apstrakt

**Uvod/Cilj.** Akutno trovanje etanolom čini značajan deo akutne patologije izazvane toksičnim hemikalijama, a koja zahtevaju medicinsko zbrinjavanje u specijalizovanim zdrav-

stvenim ustanovama. Cilj studije bio je da se utvrde osnovne karakteristike pacijenata lečenih zbog akutnog trovanja etanolom, kao što su: prosečna starost, pol, stepen težine trovanja, smrtnost, učešće u ukupnom broju pacijenata zbrinutih zbog akutnog trovanja hemijskim agensima i odnos

ambulantno i bolnički zbrinutih bolesnika. **Metode.** Peto-godišnja retrospektivna studija slučaja je obuhvatila 20891 akutno otrovanog pacijenta u periodu 2011–2015. godina, od čega je 10731 zrinut zbog akutnog trovanja etanolom. Svi pacijenti zadovoljavali su dijagnostičke kriterijume trovanja prema Međunarodnoj klasifikaciji bolesti, 10 revizija, propisanoj od strane Svetske zdravstvene organizacije i Standardizovanoj skali težine trovanja. **Rezultati.** Praćeni parametri analizirani su kod 10731 pacijenta akutno otrovanog etanolom, a njihov prosečan broj u toku jedne studijske godine iznosio je  $2146,2 \pm 437,95$ . Prosečan broj zbrinutih pacijenata zbog akutnog trovanja etanolom ( $51,47 \pm 2,86\%$ ) bio je statistički značajno veći od prosečnog broja zbrinutih zbog akutnih trovanja uzrokovanih drugim agensima ( $p < 0,001$ ). Većina otrovnih pacijenata bile su osobe muškog pola, životne dobi od 19 do 40 godina i 41 do 65 godina, sa blagim do umerenim simptomima akutne intoksikacije etanolom, pri čemu je najviše pacijenata akutno otrovanih etanolom imalo skor težine trovanja 1 ( $70,25 \pm 5,04\%$ ,  $p < 0,001$ ). Veoma mali broj akutno otrovanih etanolom zahtevao je hospitalno lečenje ( $1,05\%$ ). Pretpostavljeni broj pacijenata sa akutnim trovanjem etanolom imao je visok procenat povećanja za razdoblje od 2016. do

2020. godine (nešto više od 20%), što je zabrinjavajuće, prvenstveno zbog njihovog visokog apsolutnog broja. **Zaključak.** Akutno trovanje alkoholom predstavlja značajan deo patologije u ukupnom broju bolesnika koji se leče zbog akutnog trovanja različitim hemijskim materijama. Rezultati ove analize pokazali su da su određene populacione grupe posebno osetljive na zloupotrebu alkohola do nivoa koji zahteva zdravstveno zbrinjavanje (muška populacija, životne dobi od 19 do 65 godina). Samo mali broj (1% svih bolesnika lečenih nakon akutnog trovanja alkoholom) zahteva klinički tretman. Dobijeni podaci pružili su osnovu za ciljano preventivno delovanje u određenim populacionim grupama, kao i odgovarajuće planiranje angažovanja profesionalnog medicinskog osoblja, materijalnih, prostornih i drugih kapaciteta. Takođe, oni predstavljaju i osnovu za dodatna detaljna društvena, ekonomska, zdravstvena i druga istraživanja u ovoj oblasti, kao i detaljniju registraciju ove vrste trovanja i uspostavljanje sistema praćenja, kao i baze podataka.

#### **Ključne reči:**

**etanol; trovanje alkoholom; srbija; demografija; faktori rizika.**

## **Introduction**

The development of science, technology and industry leads to the development and production a lot of new chemicals and their broad use creates the possibility for potential acute poisoning, which represents one of the important causes of morbidity and mortality in many countries<sup>1</sup>. According to the available data, around 355,000 persons dies in the world every year as a result of the unintentional poisoning<sup>2</sup>. Causes of poisoning, gender and age structure, severity of poisoning, as well as the outcome of treatment are specific and different among local regions within countries, as well as among countries and regions all over the world<sup>3–11</sup>. More precise data can be obtained only in those countries that have developed health information system and the exact records of illnesses and causes of death. Acute intoxications by chemical substances are very important in terms of the organization of care for emergency cases in Serbia<sup>12–15</sup>. As a part of the emergency pathology, patients requiring medical treatment and observation after acute intoxication caused by different chemical substances participate with about 8% of the total number of treated patients in the Center of the Emergency Care of the Military Medical Academy in Belgrade.

Ethanol, ethyl alcohol or alcohol, is one of the most frequently used substances of abuse all over the world. There are many reasons for that and among the most important ones are: easy availability, low price, certain types of customs, aggressive advertisements, limited legal sanctions, etc. Excessive and frequently use of alcohol often leads to the emergence of alcoholism, as well as many health and social problems<sup>16,17</sup>. About two thirds of adult population in the United States of America (USA) consume alcohol drinks, and about 10% of them in the case-history have excessive

alcohol consumption. A particular area of concern is the fact that a certain number of these acute intoxication has death as a final outcome<sup>18</sup>. According to the World Health Organization (WHO) data around 208 million of people consumed alcohol during the year 2010 that was about 4.10% of all world population aged over 15 years<sup>19,20</sup>. Also, according to the WHO data, Serbia is at a very high, 12th place, with registered 12.6 liters of consumed alcoholic beverages *per capita* during one year in population older than 15 years. Ethanol is very often cause of acute intoxication which requires an urgent care in the health care institutions.

Assessment of the frequency of an acute ethanol intoxication, gender and age of poisoned patients, severity of poisoning, as well as a level of therapeutic measurements are not fully investigated, but they are of great importance for preventive work and capacity planning (material, human resources, spatial and other capacities) necessary for medical treatment.

The aim of this study was to estimate the frequency of ethanol intoxication and to assess the basic characteristics of the patients treated due to acute ethanol poisoning including age, gender, severity of poisoning and modality of treatment. These results may be important for further prospective investigation and adequate preventive intervention in certain age and gender population, as well as for necessary staff and hospital capacities planning.

## **Methods**

### *Study design*

This study was done in the National Poison Control Centre (NPCC), Military Medical Academy (MMA), Belgrade, Serbia, which usually provides medical treatment for 4,000 to 4,500 acutely poisoned patients *per year*.

A five-year retrospective study was done in a period from 2011 to 2015. It included the patients admitted to the emergency unit due to suspected poisoning. All poisoning cases were classified according to the WHO's International Classification of Diseases-10 (ICD-10) <sup>21</sup>. The medical records were reviewed for all patients with acute ethanol intoxication (10,731 cases). They were provided with the necessary initial diagnostic procedures at the admission [history, physical examination, electrocardiogram, vital signs, laboratory and toxicological tests, and in some specific situations, with other diagnostic procedures – radiography (X-ray), computed tomography, etc.] and therapy treatments.

In accordance with their age, patients were divided into following groups: the group 1: ≤ 18 years; the group 2: 19-40 years; the group 3: 41-65 years; the group 4: > 65 years and the group 5: patients with undetermined age.

#### *Poison Severity Score*

For determining the severity of the poisoning the standardized Poison Severity Score (PSS) was used: PSS 0 - no symptoms and signs related to poisoning; PSS 1 - mild or spontaneously resolving symptoms; PSS 2 - moderate, pronounced or prolonged symptoms, PSS 3 - severe or life-threatening symptoms and PSS 4 - with lethal outcome <sup>22, 23</sup>.

#### *Ethical approval*

According to the Medicines and Medical Devices Agency of Serbia on Researches Involving Human Subjects, a retrospective research/patient file research does not fall under the scope of the Ministry of Health of the Republic of Serbia. Therefore, ethical approval by the institutional medical ethical review board (the Ethics Committee of the Military Medical Academy) was not needed.

This research was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. For this type of study, a formal informed consent is not required.

#### *Statistical analysis*

The complete statistic data processing was performed in the IBM SPSS Statistics 19.0 computer program (IBM,

USA, 2011). All continuous variables were represented in the form of the mean ± standard deviations (SD), while the categorical variables were represented with the percentage of certain category frequency. For categorical variables the statistical significance of differences was examined by chi-square ( $\chi^2$ ) test. Ratios between variables were tested by Pearson's correlation coefficient. All the analyses were evaluated at the level of statistical significance of  $p < 0.05$ . Health forecasting, as a novel area of forecasting, is a valuable tool for predicting future health events or situations such as demands for health services and healthcare needs. For purpose of our study, exponential smoothing (dampened trend) method was chosen for number of patient's time series prediction.

### **Results**

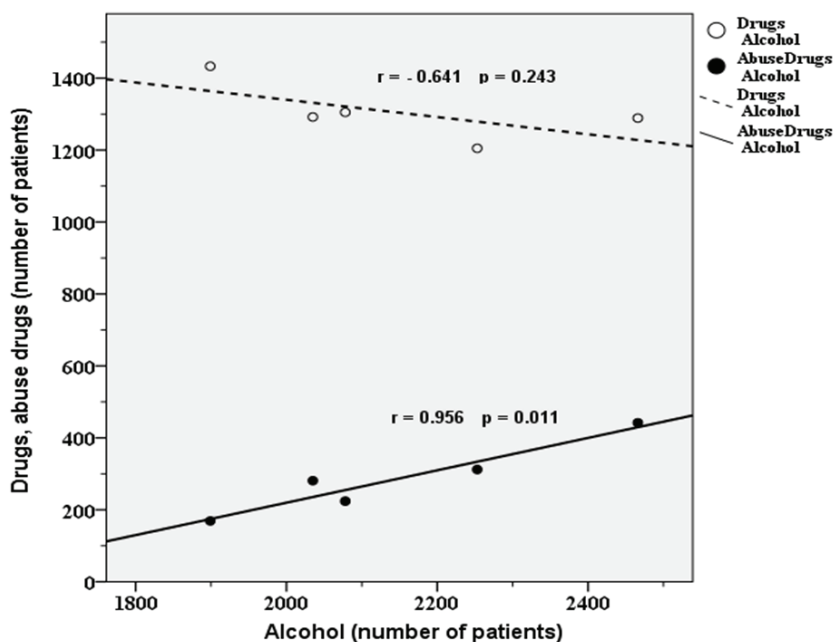
In a period from year 2011 to 2015, 20,891 patients intoxicated by different types of chemical agents were treated (average number per year was  $4,178.8 \pm 606.51$ ) including 10,731 patients treated due to acute ethanol poisoning ( $2,146.2 \pm 437.95$  per year). The share of ethanol intoxicated patients in the total number of patients did not differ significantly over the observed years, the average was  $51.47 \pm 2.86\%$ . Their number was statistically significantly higher ( $p < 0.001$ ) in comparison to the other poisoned patients (Table 1).

The number of patient intoxicated by ethanol significantly positively correlated with the number of patient poisoned by abuse drugs ( $r = 0.956$ ;  $p < 0.05$ ). There was no positive correlation with number of patients poisoned by prescribed drugs ( $r = -0.641$ ; n.s.) (Figure 1).

Generally, the frequency of intoxication was greater in male than in female population due to high prevalence of ethanol poisoning. In the male population, a slight increasing trend of patients who were treated due to intoxication by ethanol, as well as by other agents was evident. The increase in the number of patients treated due to acute alcohol poisoning was higher than the number of patients treated after poisoning with other agents ( $p < 0.001$ ). In the female population, a statistically significant increase of acute ethanol poisonings in comparison with the number of treated patients after poisoning by other chemicals was evident ( $p < 0.001$ ).

**Table 1**  
**The total number of patients poisoned by different chemicals in the period from year 2011 to 2015**

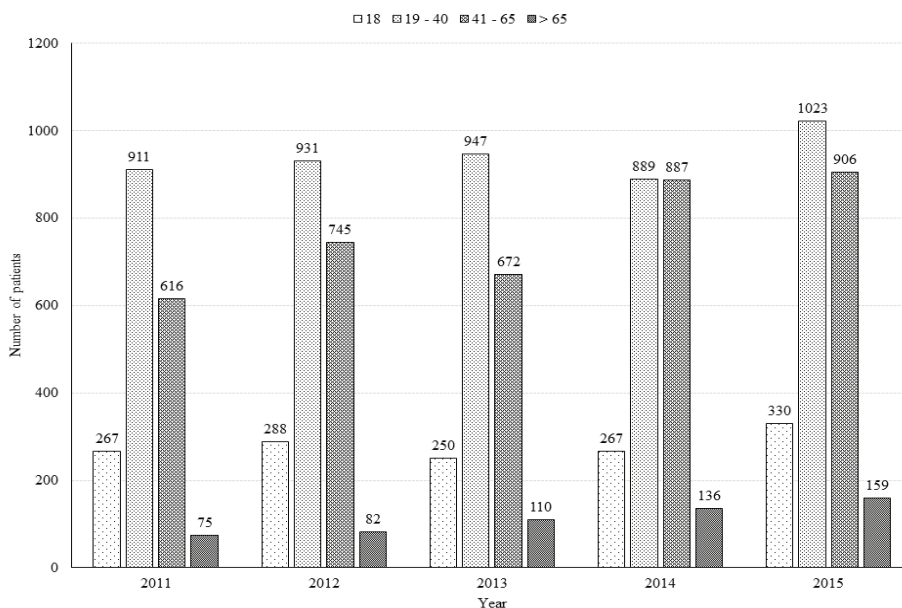
Agents	Number of patients <i>per year</i>					Total/average number
	2011	2012	2013	2014	2015	2011–2015
Alcohol	1,899	2,078	2,035	2,253	2,466	10,731/2,146.20
Drugs	1,433	1,305	1,292	1,205	1,289	6,524/1,304.80
Substances of abuse	169	224	281	312	442	1,428/285.60
Gas/Vapors	120	144	173	176	189	802/160.40
Corrosives	59	82	83	120	97	441/88.2
Pesticides	58	97	81	89	62	387/77.40
Fungi/Plants	21	57	53	64	32	227/45.40
Other	73	76	76	64	62	351/70.20
<b>Total</b>	<b>3,832</b>	<b>4,063</b>	<b>4,074</b>	<b>4,283</b>	<b>4,639</b>	<b>20,891/4,178.80</b>



**Fig. 1 – Correlation of the total number of patients poisoned by alcohol and drugs, or drugs of abuse in the period from year 2011 to 2015.**

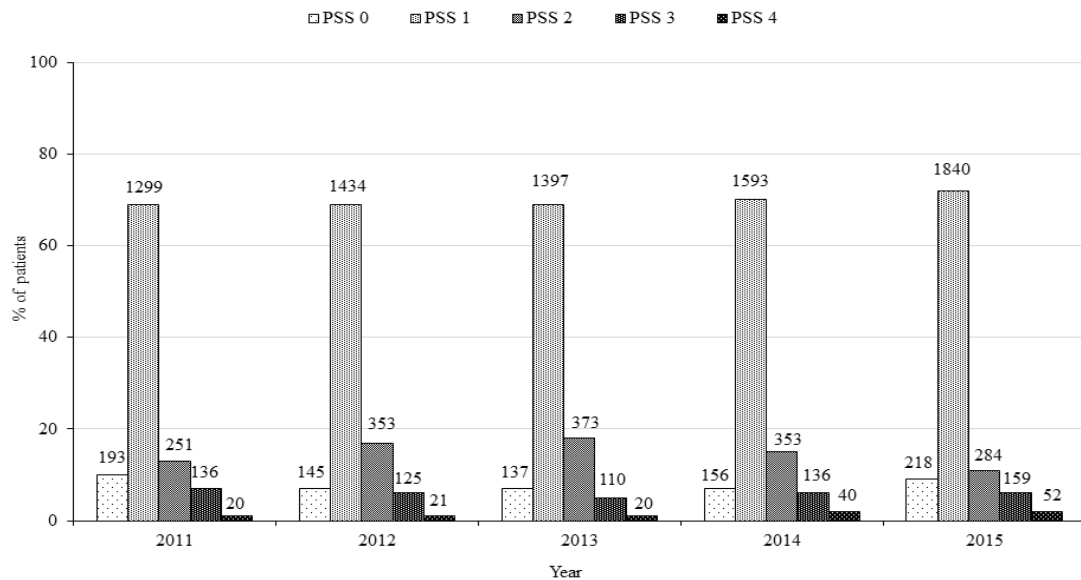
Most of patients intoxicated by ethanol were aged from 19 to 40 years, and their number was significantly higher in comparison with other age groups of poisoned patients ( $p < 0.001$ ). The total number of the patients poisoned by ethanol was significantly lower in the following groups: the group  $\leq 18$  years ( $13.08 \pm 1.94\%$ ), the group  $> 65$  years ( $5.16 \pm 2.32\%$ ) and the group of unknown age ( $2.49 \pm 1.76\%$ ). Figure 2 shows a reduction in the number of ethanol intoxicated patients aged from 19 to 40 years during 2014, and then, in 2015, their significant growth. In the group of patients aged from 41 to 65 years, the reduction of the number of poisonings in 2013 year was registered, while in

2014 and 2015 a significant increase in the number of patients poisoned by ethanol was observed. In the group of patients poisoned by other chemicals, the average number of treated patients was as follows: in the group of  $\leq 18$  years  $10.12 \pm 1.80\%$ , in the group of 19–40 years  $47.43 \pm 3.93\%$ , in the group of 41–65 years  $34.04 \pm 3.02\%$ , in the group of more than 65 years  $6.81 \pm 0.81\%$  and in the group in which the age was not precisely determined  $1.61 \pm 1.01\%$ . Comparison of age distribution between patients acutely intoxicated by ethanol and those acutely intoxicated by other chemicals, revealed a statistically significant difference ( $p < 0.001$ ) only between groups of patients aged 41 to 65 years during 2014 and 2015 year.



**Fig. 2 – Annual age distribution of patients poisoned by ethanol in the period from year 2011 to 2015.**





Numbers above columns represent absolute number of patients regarding PSS.

**Fig. 3 – Distribution of ethanol poisonings by the Poison Severity Score (PSS).**

**Table 2**

**Predicting number of patients (five year period) regarding causal agents**

Agents	Forecast number <i>per year</i> (LCL-UCL)				
	2016	2017	2018	2019	2020
Alcohol	2,532 (2,038-3,027)	2,663 (2,159-3,167)	2,794 (2,281-3,307)	2,925 (2,402-3,447)	3,056 (2,524-3,587)
Drugs	1,197 (811-1,583)	1,158 (759-1,558)	1,120 (707-1,532)	1,081 (656-1,506)	1,043 (606-1,480)
Substances of abuse	473 (301-645)	537 (361-712)	600 (421-779)	663 (480-846)	727 (540-913)
Gas/Vapors	210 (155-265)	227 (160-285)	244 (183-305)	260 (197-324)	277 (211-344)
Corrosives	121 (25-217)	133 (33-232)	144 (41-247)	155 (49-262)	166 (57-276)
Pesticides	77 (0-191)	76 (0-192)	76 (0-194)	76 (0-196)	76 (0-199)
Fungi/Plants	44 (0-166)	44 (0-168)	44 (0-170)	44 (0-172)	44 (0-174)
Other	61 (34-87)	57 (30-84)	54 (27-81)	50 (23-78)	47 (20-74)

**Forecast – predicting number of patients; LCL – lower confidence limit; UCL – upper confidence limit.**

Analysis of the severity of poisoning by ethanol showed that the highest number of patients had PSS 1 (70.25 ± 5.04%,  $p < 0.001$ ), while the rest of the severity of poisoning were represented in the significantly lower percentages: PSS 0 – 13.00 ± 2.07%, PSS 2 – 14.71 ± 4.57%, PSS 3 – 6.03 ± 5.48% and PSS 4 – 0.02 ± 0.07%. In 1.39 ± 1.25% of the patients the severity of poisoning was not registered (Figure 3).

The average number of patients hospitalized due to acute ethanol intoxication (after initial treatment in the Emergency room) was 1.05 ± 0.62% and it was significantly lower in comparison with poisoning by all other groups of agents ( $p < 0.001$ ). During the entire period observed, a significant increase in the trend of hospitalized patients after acute ethanol poisoning was registered in 2014. Of the total number of hospitalization *per year* due to acute poisoning, ethanol one was the reason in only 0.35 ± 0.17% of patients ( $p < 0.001$ ).

The number of patients treated for acute ethanol poisoning showed the trend of increasing, so that at the end of the observation period it was higher than the number of patients poisoned by all other chemicals. With intention to predict future poisoning trends and healthcare needs, exponential smoothing (dampened trend) method was used. Table 2 shows the predicted number of poisonings by the type of causal agents. The largest increase was registered in the category of poisoning by substances of abuse, followed by ethanol. The minimal growth trend was registered in the categories of corrosives and vapours, while in other categories declining trend was registered.

Graphical presentation of registered cases of poisoning by categories (for the period from 2011 to 2015), as well as the projected trends for the same categories of agents (for the period from 2016 to 2020) are shown in Figure 4.

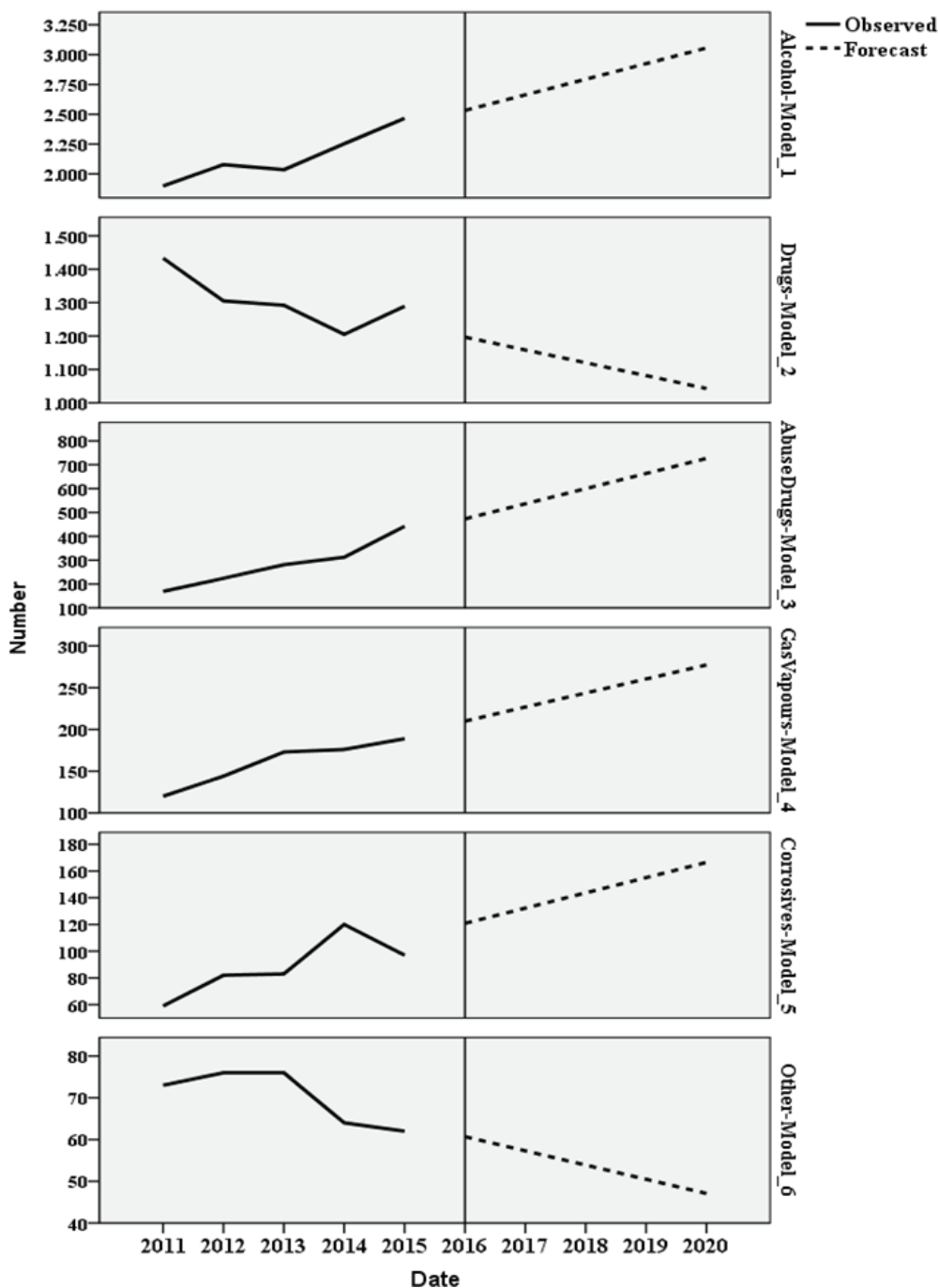


Fig. 4 – Graphic presentation of historical data (2011–2015) and forecasting (2016–2020) (agents without increasing or decreasing trend are excluded).

## Discussion

Ethanol is the most common psychoactive substance used by adolescents and adults in Serbia<sup>24–26</sup> and is one of the most commonly abused substances in the world<sup>27</sup>. Excessive and uncontrolled consumption of ethanol greatly increases the risk of trauma, especially trauma due to motor vehicle collisions or violent crimes. Excessive consumption of ethanol results in serious health and social consequences such as acute drunkenness, chronic alcoholism and dependence

with all its consequences. Alcohol causes 1.8 million deaths (3.2% of total) and a loss of 58.3 million (4% of total) of Disability-Adjusted Life Years (DALY)<sup>2</sup>. Unintentional injuries alone account for about one third of 1.8 million deaths, while neuro-psychiatric conditions account for close to 40% of 58.3 million DALYs. The burden is not equally distributed among countries. In Europe alone, alcohol consumption was responsible for many deaths among young people<sup>28</sup>.

In a study of Meropol et al.<sup>29</sup>, in which relationship between alcohol abuse injuries in a population of adolescents

treated in the emergency department was investigated, the highest percentage of positive ethanol test results was registered in a group of 295 young patients treated at an urban trauma center (15% of total subjects, and 22% of subjects aged 17–21 had positive ethanol test).

Also, in the Clinic for Emergency Assistance in Oslo (Oslo study) 2,343 adult patients have been treated after overdose by substances of abuse during one-year period, and 55.10% (1,291) of these patients were treated due to acute ethanol poisoning<sup>30</sup>. In our study, the average number of treated patients after acute ethanol poisoning in comparison to the total number of all treated patients after acute poisonings was 51.47%. In the Oslo study<sup>30</sup>, more than 89.00% of all patients were treated after excessive consumption of ethanol, drugs, as well as substances of abuse. In our study, share of patients with acute intoxication by drugs, substances of abuse and ethanol was almost equal - 89.43%. Comparing the total numbers of poisoning from the Oslo study with those treated in our centre, and taking into account the number of Oslo inhabitants of 900,000 and Belgrade of about 1,660,000, the incidence was almost the same - 0.26% and 0.25%, respectively.

By comparing gender representation in both studies, the results are as follows: in our study the relationship between the male and female patients treated for acute ethanol poisoning was 77.40%: 22.60%, respectively, and in the Oslo study it was 68.29%:31.71%, respectively<sup>30</sup>. We can conclude that sex representation is also very similar in both studies - the male subjects are more often treated for acute ethanol poisoning.

The Oslo study used different criteria for classification by age. Patients were divided into three groups: the group I ≤ 25 years, the group II 26–50 years and the group III > 50 years. In this study over than 52% of treated patients were in the group of 26–50 years<sup>30</sup>. In our study, the highest number of treated patients after acute ethanol poisonings was observed in the age groups from 19 to 40 years (44.03%) and from 41 to 65 years (35.47%). In both studies is evident that these patients belonged to a working population. It specifically indicates the possible socio-economic implications and the need for more active prevention work in these population groups.

The severity of poisoning was assessed by the different criteria: Glasgow coma scale in the Oslo study and PSS in our study. Both studies showed that most of the treated patients did not had severe clinical signs of poisoning. The first study showed that most of treated patients (85.19%) were in the group with Glasgow coma scale of 10–15. Our study showed that in a population acutely poisoned by ethanol 77.43% of patients were in the group with PSS score ≤ 1. Due to the different criteria which define scores used, it was difficult to perform accurate comparison, but it is

evident that in both studies the proportion of patients with mild to moderate level of poisoning severity was high.

In accordance with severity of poisoning, only 1.05% of our patients intoxicated by ethanol, after initial treatment in the Emergency department required additional hospital treatment. At the same time, this group of patients was only 0.35% ± 0.17% of all poisoned and treated patients in the observed period. This data is very important for the planning of adequate spatial capacities primarily in an emergency service, as well as the planning and scheduling of staff engagement.

Another important planning factor is estimated number of patients in the future. Our forecast for the period 2016–2020 indicates the highest increase of poisoning by substances of abuse (about 40%). However, predicted increase of slightly more than 20% for acute ethanol intoxicants, may represent the greatest burden for emergency due to high absolute number of patients.

### Conclusion

Acute alcohol poisoning represents a significant part of the pathology in the total number of patients treated for acute poisoning by various chemical substances. The results of this study showed that certain populations are particularly vulnerable to abuse of alcohol to the level requiring health care (male population, aged 19–65 years). Most of patients had mild signs and symptoms of poisoning and required only supportive and symptomatic treatment in the emergency room.

The presented data provide the basis for a more targeted preventive action in certain population groups, as well as the adequate planning of professional medical staff, material, spatial and other capacities. Given the potential impact of excessive alcohol consumption on the national health, along with the monitoring of chronic alcohol abuse, more detailed data on acute ethanol intoxication should be obtained. Enhancement of the monitoring system and database should enable the dissemination of data and information on trends in alcohol consumption, actual epidemiological situation regarding alcohol drinking, including alcohol-related mortality and possible details of health policy responses.

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## Influence of secondary hyperparathyroidism in management of anemia in patients on regular hemodialysis

Uticaj sekundarnog hiperparatireodizma na lečenje anemije kod bolesnika na hroničnom programu hemodijalize

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### Abstract

**Background/Aim.** Anemia is a common complication in hemodialysis patients. Treatment of anemia is affected by iron deficiency, insufficient dose of erythropoietin, microinflammation, vitamin D deficiency, increased intact parathyroid hormone concentration and inadequate hemodialysis. The aim of the study was to determine the prevalence of vitamin D deficiency and its impact on hemoglobin concentration, iron status, microinflammation, malnutrition, dialysis adequacy and erythropoietin dose in patients on regular hemodialysis. **Methods.** The study involved 120 patients divided into three groups: severely deficient of vitamin D: 25-hydroxyvitamin D [25(OH)D] < 10 ng/mL; deficient – 25(OH)D within range of 10–20 ng/mL, and insufficient – 25(OH)D > 20 ng/mL. For statistical analysis Kolmogorov-Smirnov test, the single-factor parametric analysis of variance – ANOVA and Kruskal-Wallis test were used. **Results.** The prevalence of vitamin D deficiency in patients on regular hemodialysis was 75.83%, while the prevalence of severe

vitamin D deficiency was 24.7%. Patients with severe vitamin D deficiency had lower blood concentration of hemoglobin, hematocrit, serum concentration of total proteins and albumin, and dialysis indices were also lower compared to the other two groups of patients. The level of C-reactive protein was significantly higher in the group of patients with severe vitamin D deficiency than in the two rest groups. **Conclusion.** Hemodialysis patients with severe vitamin D deficiency have lower hemoglobin, lower dialysis adequacy, significant microinflammation, malnutrition, bone metabolism disorders and need higher dose of erythropoietin than patients whose vitamin D was higher than 10 ng/mL. Vitamin D is important risk factor for development of anemia in hemodialysis patients and important factor that can affect treatment of anemia in these patients.

**Key words:**  
anemia; erythropoietin; renal dialysis; risk factors; vitamin d.

### Apstrakt

**Uvod/Cilj.** Anemija je česta komplikacija kod bolesnika na hemodijalizi. Na lečenje anemije utiču: nedostatak gvožđa, nedovoljna doza eritropoetina, mikroinflamacija, nedostatak vitamina D, povećana koncentracija intaktnog paratireoidnog hormona i neadekvatna hemodijaliza. Cilj rada bio je da se utvrdi prevalenca nedostatka vitamina D, kao i njegov uticaj na koncentraciju hemoglobina u krvi, status gvožđa, mikroinflamaciju, malnutriciju, adekvatnost hemodijalize i dozu eritropoetina kod bolesnika na redovnoj hemodijalizi. **Metoda.** Ispitivanjem je bilo obuhvaćeno 120 bolesnika podeljenih u tri grupe: grupa sa teškim deficitom vitamina D

– koncentracija 25-hidroksi vitamina D [25(OH)D] < 10 ng/mL; grupa sa deficitom vitamina D – koncentracija 25(OH)D 10–20 ng/mL; grupa sa nedovoljnim nivoom vitamina D – koncentracija 25(OH)D > 20 ng/mL. Za statističku analizu korišćeni su: Kolmogorov Smirnov test, jednofaktorska parametarska analiza varijanse-ANOVA i Kruskal-Wallis-ov test. **Rezultati.** Prevalenca snižene koncentracije 25(OH)D u serumu bolesnika koji su se lečili redovnom hemodijalizom iznosila je 75,83%, a prevalenca teškog nedostatka vitamina D 24,17%. Bolesnici sa teškim deficitom vitamina D u serumu imali su statistički značajno nižu koncentraciju hemoglobina u krvi, hematokrita, koncentraciju ukupnih proteina i albumina u serumu i vrednosti

parametara adekvatnosti hemodijalize. Koncentracija C-reaktivnog proteina u serumu bila je statistički značajno viša kod bolesnika sa teškim deficitom vitamina D u serumu, u odnosu na bolesnike sa koncentracijom 25(OH)D u serumu  $\geq 10$  ng/mL. **Zaključak.** Bolesnici sa teškim deficitom vitamina D u serumu imaju manju koncentraciju hemoglobina u krvi, manje adekvatnu hemodijalizu, značajnu mikroinflamaciju, malnutriciju, poremećaj metabolizma koštanog

tkiva i zahtevaju veću dozu eritropoetina u odnosu na bolesnike sa koncentracijom 25(OH)D  $\geq 10$  ng/mL. Vitamin D je značajan faktor rizika od razvoja i lečenja anemije kod bolesnika na redovnom programu hemodijalize.

**Ključne reči:**  
**anemija; eritropoetin; hemodijaliza; faktori rizika; vitamin d.**

## Introduction

Chronic kidney disease (CKD) is a progressive disease and loss of renal function is followed by various complications of which anemia, secondary hyperparathyroidism (SHPTH) and cardiovascular diseases are the most important<sup>1,2</sup>. Ninety percent of patients starting with hemodialysis are diagnosed with anemia caused by lack of endogen erythropoietin (EPO) which stimulates erythropoiesis in the bone marrow<sup>3</sup>. Among other causes of anemia the most important is hemorrhage<sup>3</sup>. Screening for anemia should be performed when glomerular filtration rate (GFR) is less than 60 mL/min/1.73 m<sup>2</sup>. Measured parameters include hemoglobin concentration (Hb), hematocrit (Hct), red blood cell indices, serum iron (Fe<sup>2+</sup>) and ferritin concentration (FER), transferrin saturation (TSAT) and serum concentration of C-reactive protein (CRP)<sup>1-3</sup>.

Anemia is independent risk factor for CKD progression and cardiovascular complications<sup>4-10</sup>. In hemodialysis patients treatment of anemia with EPO should be set when Hb concentration is less than 100 g/L while target Hb concentration should be within range 100–120 g/L<sup>11</sup>. Prior to treatment with EPO optimum iron status in hemodialysis patients should be ensured (TSAT = 20–40% and FER = 100–500 ng/mL)<sup>12-15</sup>. After applying EPO the target Hb concentration in the blood is not achieved in 10–20% of patients. The risk factors that influence the treatment of anemia in hemodialysis patients include: iron deficiency, insufficient dose of EPO, microinflammation, malnutrition, the lack of vitamin D, SHPTH, inadequate hemodialysis, and the existence of antibodies on EPO<sup>16-21</sup>.

SHPTH is a common and significant complication in hemodialysis patients. The lack of vitamin D, the reduced production of the active metabolite of vitamin D [1,25(OH)D], hypocalcaemia and hyperphosphatemia are the main causes of the development of SHPTH in these patients<sup>22</sup>. The risk factors that reduce the production of vitamin D in hemodialysis patients include: reduced vitamin D synthesis in the skin, reduced filtration and reabsorption of vitamin D in epithelial cells of proximal renal tubules, reduced intake of vitamin D-rich foods, reduced absorption of vitamin D from the gastrointestinal tract<sup>22</sup>. The main clinical consequences of vitamin D deficiency are: development of SHPTH, reduced bone density and increased risk of fractures, reduced iron availability for Hb synthesis in erythrocytes (“functional” iron deficiency), reduced response to EPO, atherosclerosis, hypertrophy of the left heart ventricle, vascular calcification, cognitive impairment, progressive loss of residual renal

function and increased mortality rate<sup>22</sup>. The potential mechanisms of the impact of vitamin D deficiency on the development of anemia include increased production of proinflammatory mediators in the cells of the immune system: interleukin (IL)-1, IL-6, interferon- $\gamma$ , tumor necrosis factor alpha (TNF $\alpha$ ). Proinflammatory mediators block proliferation and differentiation of erythrocyte precursor cells in the bone marrow, and IL-6 stimulates formation of hepcidin in liver, which has been proved to cause a “functional” iron deficiency<sup>22,23</sup>. SHPTH causes anemia through both direct and indirect ways. The direct effects of intact parathyroid hormone (iPTH) include: blocking the formation of endogenous EPO, blocking the proliferation and differentiation of erythroid progenitors (EP) and the shortened erythrocyte life span. Indirect effects of PTH have been mainly based on the inducing revival of the bone marrow (the loss of erythrocyte precursor cells)<sup>22,23</sup>.

The aim of this study was to determine the prevalence of anemia, disorders of the metabolism of minerals, vitamin D and PTH, as well as to examine the effect of vitamin D deficiency and enhanced secretion of iPTH on the blood concentration of Hb, the status of iron, microinflammation, malnutrition, the adequacy of hemodialysis and EPO dose in patients on regular hemodialysis.

## Methods

This study included patients of the Center for Nephrology and Dialysis, Clinic for Urology, Nephrology and Dialysis, Clinical Center Kragujevac, Kragujevac, Serbia. The study was in compliance with the principles of the Declaration of Helsinki and was approved by The Ethics Committee of the Clinical Center Kragujevac. All patients involved in the study signed informed consent prior to enrollment. All examined patients were treated using bicarbonate hemodialysis 12 h per week for period longer than three months on hemodialysis machines, the type Fresenius and the type Gambro. Ultrapure dialysis fluid and high-flux as well as low-flux polysulfone dialysis membranes were used. Patients with active proved infections were not included in the study.

In order to evaluate impact of SHPTH in management of anemia in hemodialysis patients the following parameters were measured: Hb, Hct, FER, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), TSAT, calcium (Ca<sup>2+</sup>), inorganic phosphate (PO<sub>4</sub><sup>3-</sup>), alkaline phosphatase (ALP), vitamin D and iPTH. Parameters of hemodialysis adequacy were also considered.

Serum samples from patients were collected prior to hemodialysis and prior to heparin administration. Every laboratory parameter was assigned with the value that was the mean of two measuring in two successive months.

Total Hb was measured using colorimetric method. The target Hb level in patients on dialysis was 100–120 g/L.

Nutritional status of the patients was assessed by measuring total protein (TP) and albumin (Alb) concentrations in the serum, as well as by calculating the body mass index (BMI) and normalized protein catabolic rate (nPCR).

The normalized protein catabolic rate that reflects daily dietary protein intake in hemodialysis patients was calculated using formula of the National Cooperative Dialysis Study:  $nPCR = (PCR \times 0.58)/Vd$ . Formula for calculating PCR is  $PCR = 9.35 G + 0.29 Vd$ , where  $G$  – urea production rate,  $Vd$  – volume of body fluid [ $Vd = 0.58 \times \text{body weight (BW)}$ ]. Urea production rate was calculated by formula  $G = [(C1 - C2)/Id] \times Vd$ , where  $C1$  is serum urea concentration prior to dialysis (mmol/L),  $C2$  – serum urea concentration after dialysis (mmol/L),  $Id$  – time (h) between two successive dialysis. Normal range for nPCR is  $1.1 \pm 0.3$  g/kg/day.

Serum concentration of iron, FER, TIBC,  $Ca^{2+}$ ,  $PO_4^{3-}$  and CRP were measured using Beckman Coulter AU680 analyzer.

Serum iron was determined by photometric method using TPTZ [2,4,6-Tri-(2-pyridyl)-5-triazine] as the chromogen. Serum iron reference range is 6.6–26.0  $\mu\text{mol/L}$ . TIBC was done indirectly by the Unsaturated Iron Binding Capacity (UIBC) method. TIBC reference range is 48–56  $\mu\text{mol/L}$ . TSAT was calculated using formula  $TSAT = Fe/TIBC \times 100\%$ . Reference range for TSAT in hemodialysis patients is 20–40%. UIBC was measured using spectrophotometric method. Reference range for UIBC is 28–54  $\mu\text{mol/L}$ . A method for FER was turbidimetric one. FER reference range in the patients underwent regular hemodialysis is 100–500 pg/mL.

CRP level in the serum was determined by the turbidimetric method. Normal CRP level in the serum is  $\leq 5$  mg/L. Microinflammation is defined as level of CRP in the serum higher than 5 mg/L.

$Ca^{2+}$  concentration in the serum was determined by a photometric test. Normal  $Ca^{2+}$  level in the serum is 2.20–2.65 mmol/L.  $PO_4^{3-}$  in the serum was determined by a photometric test. The normal  $PO_4^{3-}$  in the serum is 0.80–1.60 mmol/L.

Level of vitamin D in the serum was determined by a method of electrochemiluminescence, on the Cobase 411 analyser. Normal level of vitamin D in the serum is 20–40 ng/mL. In hemodialysis patients, normal vitamin D level is  $\geq 30$  ng/mL (30–80 ng/mL). A severe deficit is defined as the level of vitamin D  $< 10$  ng/mL, vitamin D deficiency exists if level is 10–20 ng/mL, and the insufficiency is defined as the level of vitamin D in the serum of 20–30 ng/mL. The level of iPTH in serum was determined by an immunoradiometric method (IRMA), on the gamma counter WALLAC WIZARD 1470. Normal level of iPTH in the serum is 11.8–64.5 pg/mL. In patients with hemodialysis the upper normal limit is 500 pg/mL.

The adequacy of hemodialysis was assessed on the basis of the single-pool index of adequacy of hemodialysis ( $Kt/V_{sp}$ ) calculated according to the Daugridas second-generation formula:

$$spKt/V = -\ln(C_2/C_1 - 0.008 \times T) + (4 - 3.5 \times C_2/C_1) \times UF/W,$$

with:  $C1$  – the value of urea before dialysis,  $C2$  – the value of urea after dialysis (mmol/L),  $T$  – duration of hemodialysis (h),  $UF$  – interdialysis yield (l),  $W$  – BW after hemodialysis (kg). According to K/DOQI guidelines, hemodialysis is adequate if single-pool adequacy of hemodialysis index ( $spKt/V$ )  $\geq 1.2$ . The degree of reducing urea (URR) index was calculated using following formula:  $URR = (1 - R) \times 100\%$ , where:  $R$  is the ratio of urea concentration in the serum after and before the hemodialysis treatment. Hemodialysis is adequate if the URR index = 65%–70%.

Depending on the level of vitamin D [25(OH)D] in the serum, the patients were divided into three groups. The first group involved the patients with the level of 25(OH)D lower than 10 ng/mL, the second group constituted patients with 25(OH)D levels within range of 10–20 ng/mL, while the third group constituted of the patients with 25(OH)D levels higher than 20 ng/mL. Depending on the serum level of iPTH, the patients were divided into three groups. The first group consisted of the patients who had the serum level of iPTH lower than 500 pg/mL, the second group constituted patients with iPTH levels within range 150–500 pg/mL, while the third group constituted of the patients with the iPTH levels higher than 500 pg/mL.

The statistical analysis was performed using the Kolmogorov-Smirnov test, the single-factor parametric analysis of variance (ANOVA) and Kruskal-Wallis test. The threshold of significance was the probability of 0.05 and 0.01.

## Results

The cross-sectional study was conducted at the the Clinic for Urology, Nephrology and Dialysis, Clinical Center Kragujevac, including the patients treated with regular hemodialysis in a period longer than three months. We examined 120 patients (75 men and 45 women), average age being  $63.15 \pm 10.39$  years, the average length of treatment with hemodialysis  $6.18 \pm 5.95$  years, and the average  $spKt/V$   $1.01 \pm 12.27$ . General patient data are shown in Table 1. Patients were treated with short-acting and long-acting EPO with a parenteral iron composition, and their average monthly doses are shown in Table 2.

The average values of parameters of the standard laboratory tests are shown in Table 3.

The prevalence of anemia ( $Hb < 100$  g/L) in the examined patients was 44.17% (53 patients). The average blood concentration of Hb, and average monthly single dose of EPO with short and long effects are shown in Table 2.

The prevalence of absolute iron deficiency in the examined patients was 4.17% (5 patients), and the prevalence of functional iron deficiency was also 4.17% (5 patients). One hundred and ten patients (91.66%) had the normal status of iron in the body. Twenty patients with normal level of iron and normal TSAT, had level of FER higher than 1,000  $\mu\text{g/L}$ . The examined patients were given parenteral iron. The average monthly single dose of parenteral iron was  $155.83 \pm 180.76$  mg.

**Table 1**  
**The characteristics of patients on regular hemodialysis (n = 120)**

Characteristics	Values
Gender (m/f), n (%)	75/45 (62.5/37.5)
Age (years), mean $\pm$ SD	63.15 $\pm$ 10.43
Duration of treatment with hemodialysis (years), mean $\pm$ SD	6.18 $\pm$ 5.98
Body mass index (kg/m <sup>2</sup> ), mean $\pm$ SD	24.68 $\pm$ 4.59
Lean body mass (kg), mean $\pm$ SD	71.46 $\pm$ 15.55
Ultrafiltration (L), mean $\pm$ SD	2475.00 $\pm$ 992.30
Residual diuresis (mL/24 h), mean $\pm$ SD	594.17 $\pm$ 710.08
Index of adequacy of hemodialysis, mean $\pm$ SD	1.01 $\pm$ 0.27
Single-pool adequacy of hemodialysis index, mean $\pm$ SD	1.01 $\pm$ 0.25
Urea reducing ratio (%), mean $\pm$ SD	61.91 $\pm$ 8.80
Primary kidney disease, n (%)	
<i>glomerulonephritis chronica</i>	12 (10.00)
<i>nephropathia hypertensiva</i>	39 (32.50)
<i>nephropathia diabetica</i>	16 (13.33)
<i>nephropathia obstructiva</i>	8 (6.67)
<i>nephropathia endemica</i>	1 (0.83)
<i>nephropathia chronica</i>	18 (15.00)
<i>pyelonephritis chronica</i>	3 (2.50)
<i>renes polycystici</i>	21 (17.50)
<i>nephritis tubulointerstitialis</i>	2 (1.67)
Comorbidities, n (%)	
hypertension	69 (57.50)
hypotension	3 (2.50)
other cardiovascular diseases	30 (25.00)
diabetes mellitus	18 (15.00)

m – male; f – female; SD – standard deviation.

**Table 2**  
**Doses of erythropoietin and intravenous iron: average monthly dose and ESA/Hb index**

Characteristics of treatment of anemia	Mean $\pm$ SD
Average monthly dose of erythropoietin	18,517.24 $\pm$ 9,442.79
short-term (IU)	
long-term ( $\mu$ g)	121.07 $\pm$ 76.90
ESA/Hb index	
short-term erythropoietin (IU/g)	191.39 $\pm$ 110.43
long-term erythropoietin ( $\mu$ g/g)	1.24 $\pm$ 0.83
Average monthly dose of intravenous iron saccharose (mg)	155.83 $\pm$ 181.52

ESA – erythropoiesis-stimulating agents; Hb – hemoglobin; SD – standard deviation.

Average BMI of examined patients was 24.68  $\pm$  4.57 kg/m<sup>2</sup>, and nPCR was 1.69  $\pm$  0.62 g/kg/day (Table 3).

The prevalence of vitamin D deficiency [25(OH)D  $\leq$  20 ng/mL] on examined patients was 75.83% (91 patients), and the prevalence of severe vitamin D deficiency [25(OH)D < 10 ng/mL] was 24.17% (29 patients). The level of 25(OH)D in the serum of 10–20 ng/mL was present in 62 (51.67%) of patients, 20–30 ng/mL in 19 (15.83%) of patients, and normal level of 25(OH)D (30–80 ng/mL) in 10 (8.33%) of the examined patients. The prevalence of SHPTH (iPTH  $\geq$  500 pg/mL) in the examined patients was 14.17% (17 patients). The biggest number of the patients (58 or 48.33%) had iPTH  $\leq$  150 pg/mL, and 45 (37.50%) patients had the serum iPTH level within range of 150–500 pg/mL. Almost all the patients – 110 (91.60%) were treated with the same Ca<sup>2+</sup>-containing PO<sub>4</sub><sup>3-</sup> binding agent, 54 (45%) of patients were treated with active vitamin D metabolites and 1

(0.83%) patient was treated with vitamin D. Parenteral form of paricalcitol was given to 14 (11.67%) of patients, and average monthly dose was 30.00  $\pm$  15.20  $\mu$ g.

The patients with 25(OH)D level in the serum of less than 10 ng/mL had a highly statistically significant ( $p < 0.01$ ) lower concentration of Hb TP, Alb and TSAT and statistically significantly lower Hct and CRP ( $p < 0.05$ ) compared to the patients with 25(OH)D level in the serum of 10–20 ng/mL and higher than 20 ng/mL (Table 4). There was no statistically significant difference between the second and third group of patients in concentrations of Hb, Hct and CRP ( $p > 0.05$ ) (Table 4). There was no statistically significant difference in average monthly dose of short-acting and long-acting EPO among the examined groups of patients (Table 5).

There was no statistically significant difference between the second and third group of patients in the level of TP and Alb and TIBC (Table 4).



**Table 3**  
**Laboratory parameters results**

Parameters	Mean ± SD
Hb (g/L)	101.79 ± 10.94
Hct (%)	30.71 ± 3.21
TP (g/L)	61.47 ± 4.95
Alb (g/L)	36.45 ± 3.51
nPCR (g/kg/dan)	1.69 ± 0.62
Fe <sup>2+</sup> (μmol/L)	10.25 ± 3.38
TIBC (μmol/L)	34.23 ± 6.36
UIBC (μmol/L)	23.95 ± 6.56
TSAT (%)	30.78 ± 10.98
F (μg/L)	790.79 ± 354.58
CRP (mg/L)	11.02 ± 19.63
Ca <sup>2+</sup> (mmol/L)	2.24 ± 0.18
PO <sub>4</sub> <sup>3-</sup> (mmol/L)	1.49 ± 0.37
Ca <sup>2+</sup> × PO <sub>4</sub> <sup>3-</sup> (mmol <sup>2</sup> /L <sup>2</sup> )	3.34 ± 0.87
ALP (mg/L)	106.51 ± 141.39
25(OH)D (ng/mL)	15.91 ± 9.68
iPTH (pg/mL)	278.70 ± 381.03

**Hb** – hemoglobin; **Hct** – hematocrit; **TP** – total protein; **Alb** – albumin; **nPCR** – normalized protein catabolic rate; **TIBC** – total iron binding capacity; **UIBC** – unsaturated iron binding capacity; **TSAT** – transferrin saturation [TSAT = (Fe<sup>2+</sup>/TIBC) × 100 (%)]; **F** – ferritin; **CRP** – C-reactive protein; **Ca<sup>2+</sup> × PO<sub>4</sub><sup>3-</sup>** – solubility product; **ALP** – alkaline phosphatase; **25(OH)D** – vitamin D; **iPTH** – parathyroid hormone.

Patients with severe vitamin D deficiency had statistically significant ( $p < 0.005$ ) lower Kt/V as well as URR compared to other groups of patient (Table 5). The two rest groups had no statistically significant difference in the parameters of hemodialysis adequacy (Table 5).

Patients with levels of iPTH > 500 pg/mL had statistically significantly higher level of ALP, a higher PO<sub>4</sub><sup>3-</sup> level, and a solubility product compared to patients with the serum levels of iPTH lower than 150 pg/mL (Tables 6 and 7).

### Discussion

Among the patients with the end-stage kidney disease who begun regular hemodialysis, 90% suffer from anemia. Main clinical consequences of CKD are: progressive loss of residual renal function, cardiovascular complications, cognitive impairment and reduced quality of life of hemodialysis patients<sup>24</sup>.

Regardless of appropriate treatment of anemia, which includes parenteral administration of iron and EPO, anemia is still a common complication in the population of patients treated with regular hemodialysis. Anemia, defined as blood Hb concentration lower than 100 g/L, had high prevalence (44.17%) in the examined patients with CKD. The most important risk factor that affect the treatment of anemia in patients on dialysis include: iron deficiency, insufficient dose of EPO, inflammation, infection, SHPTH, increased serum iPTH levels, lack of vitamin D in the serum, malnutrition and inadequate hemodialysis<sup>24</sup>.

**Table 4**

**The influence of vitamin D deficiency on the concentration of hemoglobin (Hb), C-reactive protein (CRP), parameters of nutritive status and metabolism of minerals and bone tissue in hemodialysis patients**

Parameters	Patients' groups according to 25(OH)D level (ng/mL)			Significance	
	< 10	10–20	> 20	F	p
Hb (g/L) <sup>a</sup> , mean ± SD	95.31 ± 9.42	103.77 ± 11.08	104.02 ± 9.75	7.431	0.001
Hct (%) <sup>b</sup> , mean ± SD	29.16 ± 2.88	31.20 ± 3.28	31.19 ± 2.94	4.728	0.011
CRP (mg/L) <sup>c</sup> , mean ± SD	18.42 ± 32.22	8.24 ± 9.96	9.55 ± 17.53		0.026
TP (g/L) <sup>d</sup> , mean ± SD	58.76 ± 6.18	62.28 ± 4.18	62.45 ± 4.18	6.273	0.003
Alb (g/L) <sup>e</sup> , mean ± SD	33.47 ± 4.18	37.16 ± 2.41	37.91 ± 3.11	18.493	0.0001
nPCR (g/kg/d), day	1.52 ± 0.51	1.70 ± 0.64	1.85 ± 0.66	2.112	0.126
Fe <sup>2+</sup> (μmol/L), mean ± SD	9.00 ± 2.72	10.64 ± 3.44	10.68 ± 3.66	2.686	0.072
TIBC (μmol/L) <sup>f</sup> , mean ± SD	30.98 ± 7.40	35.12 ± 5.96	35.55 ± 5.02	5.389	0.006
UIBC (μmol/L), mean ± SD	22.09 ± 7.55	24.44 ± 6.29	24.78 ± 5.82	1.584	0.209
TSAT (%), mean ± SD	30.64 ± 11.41	30.83 ± 10.55	30.84 ± 11.82	0.003	0.940
F (ng/mL), mean ± SD	775.40 ± 405.24	789.82 ± 380.04	808.26 ± 234.34	0.062	0.940
Ca <sup>2+</sup> (mmol/L), mean ± SD	2.18 ± 0.21	2.27 ± 0.15	2.24 ± 0.21	2.107	0.126
PO <sub>4</sub> <sup>3-</sup> (mmol/L)	1.43 ± 0.40	1.50 ± 0.37	1.53 ± 0.35	0.501	0.607
Ca <sup>2+</sup> × PO <sub>4</sub> <sup>3-</sup> (mmol <sup>2</sup> /L <sup>2</sup> )	3.11 ± 0.89	3.40 ± 0.84	3.44 ± 0.89	1.353	0.263
ALP (IU/L)	133.53 ± 251.15	98.42 ± 82.32	96.79 ± 78.28		0.961
iPTH (pg/mL)	278.10 ± 466.71	290.51 ± 362.75	254.06 ± 334.04		0.501

**Hct** – hematocrit; **TP** – total proteins; **Alb** – albumin; **nPCR** – protein catabolism rate; **TIBC** – total iron binding capacity; **UIBC** – unsaturated iron binding capacity; **TSAT** – transferrin saturation; **F** – ferritin; **Ca<sup>2+</sup> × PO<sub>4</sub><sup>3-</sup>** – solubility product; **ALP** – alkaline phosphatase; **iPTH** – intact parathyroid hormone; **SD** – standard deviation.

**Statistical analysis:** <sup>a</sup> –  $p_{I,II} = 0.001$ ,  $p_{I,III} = 0.001$ ,  $p_{II,III} = 1.000$ ; <sup>b</sup> –  $p_{I,II} = 0.013$ ,  $p_{I,III} = 0.043$ ,  $p_{II,III} = 1.000$ ; <sup>c</sup> –  $p_{I,II} = 0.026$ ,  $p_{I,III} > 0.05$ ,  $p_{II,III} > 0.05$ ; <sup>d</sup> –  $p_{I,II} = 0.004$ ,  $p_{I,III} = 0.011$ ,  $p_{II,III} = 1.000$ ; <sup>e</sup> –  $p_{I,II} = 0.0001$ ,  $p_{I,III} = 0.0001$ ,  $p_{II,III} = 1.000$ ; <sup>f</sup> –  $p_{I,II} = 0.010$ ,  $p_{I,III} = 0.016$ ,  $p_{II,III} = 1.000$ .

**Note:** H for CRP, ALP and iPTH are 7.266, 0.080 and 1.382, respectively.

**Table 5**  
**The influence of [25(OH)D] deficiency on parameters of hemodialysis adequacy, dose of erythropoietin and iron**

Test parameters	Patients' groups according to 25(OH)D (ng/mL)			Significance	
	< 10	10–20	> 20	F	p
Kt/V <sup>a</sup> , mean ± SD	0.91 ± 0.21	1.08 ± 0.31	0.96 ± 0.21	4.848	0.009
spKt/V, mean ± SD	0.96 ± 0.26	1.04 ± 0.26	0.99 ± 0.22	1.173	0.313
URR (%) <sup>b</sup> , mean ± SD	58.37 ± 8.30	64.09 ± 8.94	60.78 ± 7.83	4.770	0.010
ESA/Hb index – KDE, mean ± SD	215.56 ± 131.27	202.00 ± 113.29	178.07 ± 65.28	0.538	0.587
ESA/Hb index – DDE, mean ± SD	1.34 ± 0.54	1.31 ± 1.04	1.13 ± 0.72	0.224	0.801
Monthly dose – KDE, mean ± SD	19631.58 ± 10462.69	18333.33 ± 9619.69	17636.36 ± 6622.28	0.181	0.835
Monthly dose – DDE, mean ± SD	129.44 ± 52.35	125.24 ± 92.09	115.77 ± 65.66	0.095	0.910
PMDG (mg), mean ± SD	184.48 ± 223.25	129.84 ± 142.99	182.76 ± 206.27		0.496

kt/V – index of adequacy of hemodialysis; spkt/V – single pool adequacy of hemodialysis index; URR – urea reducing ratio; ESA – erythropoiesis-stimulating agents; Hb – hemoglobin; SD – standard deviation; KDE – average monthly dose of short-acting erythropoietin; DDE – average monthly dose of long-term erythropoietin; PMDG – average monthly dose of intravenous iron.

Statistical analysis: <sup>a</sup> –  $p_{I,II} = 0.014$ ,  $p_{I,III} = 1.000$ ,  $p_{II,III} = 0.134$ ; <sup>b</sup> –  $p_{I,II} = 0.011$ ,  $p_{I,III} = 0.852$ ,  $p_{II,III} = 0.263$ ;

Note: H for PMDG is 1.402.

**Table 6**  
**The influence of intact parathyroid hormone (iPTH) on the concentration of hemoglobin (Hb), C-reactive protein (CRP), parameters of nutritional status, metabolism of minerals and bone tissue, hemodialysis adequacy, erythropoietin and iron dosage**

Parameters	Patients' groups according to iPTH (ng/mL) level			Significance	
	150	150–500	> 500	F	p
Hb (g/L) <sup>a</sup> , mean ± SD	101.85 ± 10.09	101.80 ± 13.03	101.53 ± 7.07	0.006	0.994
Hct (%) <sup>b</sup> , mean ± SD	30.66 ± 2.95	30.70 ± 3.83	30.87 ± 2.03	0.028	0.972
CRP (mg/L) <sup>c</sup> , mean ± SD	11.61 ± 24.50	8.29 ± 8.50	16.23 ± 21.77	1.061	0.349
TP (g/L) <sup>d</sup> , mean ± SD	62.24 ± 5.12	60.33 ± 5.03	61.85 ± 3.35	1.977	0.143
Alb (g/L) <sup>e</sup> , mean ± SD	36.51 ± 3.65	36.42 ± 3.67	36.32 ± 2.46	0.052	0.950
nPCR (g/kg/day)	1.74 ± 0.54	1.61 ± 0.73	1.76 ± 0.53	0.652	0.523
Fe <sup>2+</sup> (μmol/L), mean ± SD	9.91 ± 2.59	11.10 ± 4.09	9.17 ± 3.25	2.657	0.074
TIBC (μmol/L) <sup>f</sup> , mean ± SD	34.30 ± 7.47	34.47 ± 4.93	33.32 ± 5.68	0.205	0.815
UIBC (μmol/L), mean ± SD	24.46 ± 6.78	23.28 ± 6.25	24.00 ± 6.66	0.406	0.668
TSAT (%), mean ± SD	29.73 ± 8.23	33.03 ± 13.13	28.41 ± 12.10	1.625	0.201
F (ng/mL), mean ± SD	768.40 ± 339.46	806.22 ± 398.23	826.35 ± 273.33	0.241	0.786
Ca <sup>2+</sup> (mmol/L), mean ± SD	2.27 ± 0.17	2.18 ± 0.17	2.26 ± 0.21	3.621	0.030
PO <sub>4</sub> <sup>3-</sup> (mmol/L), mean ± SD	1.37 ± 0.34	1.56 ± 0.38	1.72 ± 0.30	7.649	0.001
Ca <sup>2+</sup> × PO <sub>4</sub> <sup>3-</sup> (mmol <sup>2</sup> /L <sup>2</sup> ), mean ± SD	3.12 ± 0.84	3.39 ± 0.82	3.91 ± 0.80	6.014	0.003
ALP (IU/L), mean ± SD	69.34 ± 26.64	93.46 ± 39.86	267.91 ± 329.43	16.795	0.0001
25(OH)D (pg/mL), mean ± SD	15.09 ± 8.24	17.26 ± 11.26	15.12 ± 9.46	0.696	0.500

Hct – hematocrit; TP – total proteins; Alb – albumin; nPCR – protein catabolism rate; TIBC – total iron binding capacity; UIBC – unsaturated iron binding capacity; TSAT – transferrin saturation; F – ferritin; Ca<sup>2+</sup> × PO<sub>4</sub><sup>3-</sup> – solubility product; ALP – alkaline phosphatase; 25(OH)D – 25-hydroxy vitamin D; SD – standard deviation.

Statistical analysis: <sup>a</sup> –  $p_{I,II} = 0.028$ ,  $p_{I,III} = 1.000$ ,  $p_{II,III} = 0.393$ ; <sup>b</sup> –  $p_{I,II} = 0.027$ ,  $p_{I,III} = 0.002$ ,  $p_{II,III} = 0.337$ ; <sup>c</sup> –  $p_{I,II} = 0.324$ ,  $p_{I,III} = 0.003$ ,  $p_{II,III} = 0.093$ ; <sup>d</sup> –  $p_{I,II} = 1.000$ ,  $p_{I,III} = 0.0001$ ,  $p_{II,III} = 0.0001$ ; <sup>e</sup> –  $p_{I,II} = 0.0001$ ,  $p_{I,III} = 0.0001$ ,  $p_{II,III} = 1.000$ ; <sup>f</sup> –  $p_{I,II} = 0.010$ ,  $p_{I,III} = 0.016$ ,  $p_{II,III} = 1.000$ .

**Table 7**  
**The influence of intact parathyroid hormone (iPTH) on parameters of hemodialysis adequacy, erythropoietin and iron dosage**

Test parameters	Patients' groups according to iPTH (pg/mL) level			Significance	
	150	150–500	> 500	F	p
Kt/V <sup>a</sup> , mean ± SD	1.05 ± 0.23	0.95 ± 0.25	1.04 ± 0.42	1.712	0.185
spKt/V, mean ± SD	1.06 ± 0.25	0.97 ± 0.23	0.96 ± 0.29	2.031	0.136
URR (%) <sup>b</sup> , mean ± SD	63.82 ± 7.636	59.73 ± 8.90	61.13 ± 10.89	2.909	0.058
ESA/Hb index – KDE, mean ± SD	187.42 ± 113.49	228.38 ± 112.39	145.88 ± 74.75	1.686	0.195
ESA/Hb index – DDE, mean ± SD	1.29 ± 0.87	1.20 ± 0.81	1.36 ± 0.99	0.103	0.903
Monthly dose – KDE, mean ± SD	18281.25 ± 9609.25	21000.00 ± 9708.24	14500.00 ± 6989.79	1.350	0.268
Monthly dose – DDE, mean ± SD	124.71 ± 76.25	118.61 ± 74.85	130.63 ± 88.90	0.070	0.932
PMDG (mg), mean ± SD	137.07 ± 180.29	176.67 ± 196.24	164.71 ± 135.51		0.436

**Kt/V** – index of adequacy of hemodialysis; **spKt/V** – single pool adequacy of hemodialysis index; **URR** – urea reducing ratio; **ESA** – erythropoiesis-stimulating agents; **Hb** – hemoglobin; **SD** – standard deviation.

**KDE** – average monthly dose of short-term erythropoietin; **DDE** – average monthly dose of long-term erythropoietin; **PMDG** – average monthly dose of intravenous iron.

**Note:** H for PMDG is 1.659.

Results of recent clinical studies show that vitamin D deficiency plays an important role in the development of anemia in patients treated with regular hemodialysis. Vitamin D deficiency is defined as the serum level of 25(OH)D < 20 ng/mL, while severe vitamin D deficiency is defined as the serum level of 25(OH)D < 10 ng/mL<sup>24</sup>. Vitamin D insufficiency is defined as the serum level of 25(OH)D within range of 20–30 ng/mL. Normal serum level of 25(OH)D is ≥ 30 ng/mL. Target level in the serum of 25(OH)D in patients treated with regular hemodialysis is higher than 30ng/mL (30–80 ng/mL), while the level higher than 80 ng/mL can lead to toxic effects<sup>24</sup>. Prevalence of severe vitamin D deficiency in our study group was 24.17%. Vitamin D deficiency was present in 75.83% patients, vitamin D insufficiency in 15.83% (19 patients), while normal serum level of vitamin D had 8.33% patients. These results are similar with those demonstrated by former studies that showed vitamin D deficiency prevalence in hemodialysis patients of about 80%<sup>24</sup>.

Our results point to a difference in Hb concentration among groups of patients with different vitamin D levels; patients with 25(OH)D level lower than 10 ng/mL had lower blood Hb concentration than the group with serum 25(OH)D level of 10–20 ng/mL or the group with serum 25(OH)D level higher than 20 ng/mL. These two groups of patients had no difference regarding average dose of short-term and long-term EPO indicating that patients with severe vitamin D deficiency require higher dose of EPO for the treatment of anemia. Other authors have also demonstrated that vitamin D deficient hemodialysis patients have significantly lower blood Hb concentration than patients without vitamin D deficiency and require a significantly higher dose of EPO

than patients having target level of 25(OH)D<sup>24–26</sup>. Prevalence of SHPTH in our study was 14.17%. The examined groups of patients did not differ in Hb concentrations as well as in average monthly dose of short-term and long-term EPO. Patients with high levels of serum iPTH had higher level of PO<sub>4</sub><sup>3-</sup> and ALP in the serum, and also higher Ca<sup>2+</sup> × PO<sub>4</sub><sup>3-</sup>. Former studies had similar findings – patients with SHPTH had higher Ca<sup>2+</sup> × PO<sub>4</sub><sup>3-</sup> and increased risk of vascular and valvular calcifications<sup>27–30</sup>.

### Conclusion

Prevalence of vitamin D deficiency in hemodialysis patients was high – 75.83%. Severe vitamin D deficiency was present in 24.17% of patients, while normal vitamin D level had 8.33% of patients. Patients with vitamin D deficiency level lower than 10 ng/mL had significantly lower Hb concentration and adequacy of hemodialysis indices, as well as microinflammation, malnutrition and bone metabolism disorders present and needed higher dose of EPO compared to the patients with the serum 25(OH)D level higher than 10 ng/mL. Accordingly, vitamin D could be a significant risk factor for development of anemia in hemodialysis patients.

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# Significance of cytogenetic-risk categories and refined international prognostic scoring system for overall survival in primary myelofibrosis: A single-center experience

Značaj citogenetički rizičnih kategorija i prerađenog Međunarodnog prognostnog sistema za procenu ukupnog preživljavanja u primarnoj mijelofibrozi: iskustvo jednog centra

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## Abstract

**Background/Aim.** Primary myelofibrosis (PMF) is a chronic, malignant hematological disease characterized by a leucoerythroblastic blood picture, anisopoikilocytosis tear-drop-shaped erythrocytes, different degrees of bone marrow fibrosis and hepatosplenomegaly due to extramedullary hematopoiesis. Among genetic specificities of the disease, those that stand out are chromosomal aberrations in pathological, myeloid blood cells. The aim of this study was to examine the prognostic significance of clinical, hematologic and cytogenetic parameters in PMF. **Methods.** A retrospective study included 144 patients with PMF. Karyotypes were analyzed using conventional cytogenetic methods. **Results.** The chromosome examinations were successful in 126 (88%) patients and failed in the remainder ones (12%). Karyotype was abnormal in 36/126 (29%) subjects at presentation. The most frequent changes included +9, 13q- and 20q- (28%). Other abnormalities were: aberrations of chromosome 18 and 16, deletions (9q-, 12p-, 7q-, 5q-, 6q-, 8q-), tri-

somies (+1q, +8, +10, +21), monosomies (-7, -11), 3q inversion and loss of Y chromosome. We detected four novel balanced translocations in PMF: t(17;22)(q11;q13), t(15;17)(q22;q25), t(9;12)(q22;q24) and t(2;4)(q21;p16), one constitutional translocation-rob(13;14)(q10;q10) and some new karyotype anomalies – deletion of both homologues, hyperdiploidy and the coexistence of unrelated pathological clones. **Conclusion.** Chromosomal aberrations had a significant influence on overall survival of patients with PMF according to the refined cytogenetic-risk of the International Prognostic Scoring System (Refined CIPSS) ( $p = 0.004$ ). Our patients matched the pattern of chromosome aberrations usually observed in PMF but some newly registered, balanced translocations and other rare karyotype anomalies were recorded as well.

**Key words:**  
chromosome aberrations; cytogenetics; primary myelofibrosis; prognosis.

## Apstrakt

**Uvod/Cilj.** Primarna mijelofibroza (PMF) je hronična, maligna hematološka bolest koja se karakteriše leukoeritroblastnom krvnom slikom, anizopoikilocitozom eritrocita u obliku suze, različitim stepenom fibroze kostne srži i hepatosplenomegalijom usled ekstramedularne hematopoeze. Od genetičkih specifičnosti bolesti, ističu se hromozomske aberacije u patološkim, mijeloidnim ćelijama krvi. Cilj rada bio je da se ispita prognostički značaj kliničkih, hematoloških i citogenetičkih parametara u PMF. **Metode.** Retrospektivnom studijom su bila obuhvaćena 144 bolesnika sa PMF. Analiza

kariotipa vršena je konvencionalnom citogenetičkom metodom. **Rezultati.** Hromozomska analiza je bila uspešna kod 126 (88%) bolesnika, a neuspešna kod ostalih bolesnika (12%). Aberantan kariotip je bio registrovan kod 36/126 (29%) ispitanika na prezentaciji. Najčešće aberacije bile su: +9, 13q- i 20q- (28%). Druge abnormalnosti bile su: aberacije hromozoma 18 i 16, delecije (9q-, 12p-, 7q-, 5q-, 6q-, 8q-), trizomije (+1q, +8, +10, +21), monozomije (-7, -11), inverzija 3q i gubitak Y hromozoma. Otkrili smo i četiri nove balansirane translokacije u PMF: t(17;22)(q11;q13), t(15;17)(q22;q25), t(9;12)(q22;q24) i t(2;4)(q21;p16), jednu konstitucionu translokaciju - rob(13;14)(q10;q10) i neke nove anomalije kariotipa -

delecija oba, homologa hromozoma, hiperdiploidiju i koegzistenciju nepovezanih patoloških klonova. **Zaključak.** Prema citogenetički prerađenom Međunarodnom prognoznom sistemu hromozomske aberacije su statistički značajno ( $p = 0.004$ ) uticale na ukupno preživljavanje bolesnika sa PMF. Kod naših bolesnika nađene su hromozomske aberacije uobičajne za

PMF, ali su registrovane i nove balansirane translokacije, kao i druge, retke kariotipske anomalije.

**Ključne reči:**  
**hromosomi, aberacije; citogenetika; mijelofibroza, primarna; prognoza.**

## Introduction

Primary myelofibrosis (PMF) is a clonal myeloproliferative neoplasm (MPN) characterized by bone marrow fibrosis and extramedullary hematopoiesis<sup>1, 2</sup>. Patients with PMF are heterogeneous at presentation. Adverse prognostic factors include: advanced age, presence of constitutional symptoms, anemia, decreased or elevated white blood cell count, thrombocytopenia, circulating myeloblasts and the presence of clonal chromosomal aberrations<sup>3, 4</sup>.

Cytogenetic data in PMF are often scarce and inconclusive. Their prognostic relevance is limited by difficulties in obtaining good quality metaphases from the bone marrow aspirates<sup>5</sup>. However, studies reporting successful cytogenetics showed that recurrent cytogenetic abnormalities are seen in approximately one-third of patients at diagnosis and they increase in frequency over the course of disease.

The most frequent aberrations in PMF (+1q, 13q-, 20q-, +9, +8) appear in two-thirds of patients with a pathologic karyotype<sup>5, 6</sup>. Conversely, other abnormalities, such as balanced translocations, complex karyotypes, the coexistence of two or more unrelated pathological clones, are rare. In consequence, cytogenetics is one of the fundamental prognostic parameters in some current Prognostic Scoring Systems (PSSs) in PMF.

The aim this study was to examine the prognostic significance of clinical, hematologic and cytogenetic parameters in PMF.

## Methods

We presented a retrospective analysis of 144 PMF patients who were cytogenetically evaluated. The type and frequency of chromosome abnormalities were assessed at presentation. Besides typical chromosomal abnormalities encountered in PMF, we presented a number of rather rare cytogenetic findings. In addition, we tested the prognostic impact of the chromosomal aberrations on patient survival from presentation up to the completion of the study. Due to different categorization of abnormalities in different PSSs in PMF, we examined their prognostic significance by stratifying patients in accordance with the stipulations of cytogenetic (C) PSSs [CLille, CMayo, C International PSS – (CIPSS) and Refined CIPSS]<sup>3, 7-9</sup>.

Furthermore, the impact of clinical and hematological parameters on overall survival (OS) of our patients was examined by applying different PSSs (Lille, Cervantes, IPSS, Dynamic IPSS – DIPSS, Mayo and Mayo for younger patients)<sup>3, 4, 10-13</sup>.

## Patients

Between January 2004 and December 2010, 144 patients were diagnosed with PMF at the Clinic of Hematology,

Clinical Center of Serbia, Belgrade. All patients fulfilled the WHO diagnostic criteria for PMF<sup>14, 15</sup>. Clinical and laboratory data along with cytogenetic results were collected retrospectively up to 2013, when 95 (66%) of the patients were alive and 49 (34%) had died.

Bone marrow aspirates and trephine biopsies were taken with the consent of the patients or their families. The study was approved by the Ethics Committee of the Clinical Center of Serbia in Belgrade.

## Cytogenetic analysis

Conventional cytogenetic analyses were performed on metaphases obtained from unstimulated bone marrow aspirates or peripheral blood cultures, using a previously reported technique<sup>16</sup>. Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature<sup>17</sup>. Whenever possible, twenty metaphases were examined.

## Statistics

Statistical analysis of the prognostic significance of clinical and laboratory data, as well as cytogenetic status was performed using several PSSs and cytogenetic PSSs. Survival was measured from diagnosis until the last contact or death. The data are presented as median (minimum-maximum) or n (%). Survival was evaluated using Kaplan-Meier curves and the Log-rank test for group comparisons. All analyses were performed using SPSS 20.0 (IBM corp.). Probability ( $p$ ) values  $\leq 0.05$  were considered statistically significant.

## Results

Bone marrow aspirate or peripheral blood samples were taken from 144 patients with PMF [median age 65.5 years (range 28–80 years)] for cytogenetic analysis. Comprehensive clinical and laboratory evaluations were performed at diagnosis (Table 1). Karyotype analysis failed in 18/144 (12%) patients. A normal karyotype was seen in 91/126 (72%) patients including an individual with a Robertsonian translocation. Abnormal pathologic clones were spotted in the remaining 36/126 (28%) patients. The results of cytogenetic analysis for 37 patients are presented in Table 2. The most prevalent changes were +9, 13q- and 20q-, comprising 28% of the abnormal karyotypes and were found in 10 patients, either as a single abnormality or as part of a complex karyotype. Three patients had aberrations of chromosome 18. Aberrations of 16q, deletion of chromosomes 9q, 12p and 7q as well as loss of the Y chromosome were found in two patients each. In one patient (aged 76), -Y was part of the con-

stitutional karyotype, while in the other one (aged 59) it was a clonal chromosomal abnormality. Each of the following aberrations was seen in a single patient: +1q, inv(3), 5q-, 6q-, -7, +8, 8q-, +10, -11, +21.

**Table 1**  
**Clinical and hematological characteristics in 144 patients at presentation**

Parameter	Median (minimum-maximum)	n/N*
Age, years	65.5 (28-80)	144
Age ≤ 60 years	54.5 (28-60)	50/144
Sex, F/M		55/89
Hemoglobin (g/L)	124.0 (45-181)	
Hb < 100 g/L		21/144
WBC count (x10 <sup>9</sup> /L)	11.1 (1.6-83.2)	
WBC count < 4 or > 30 x 10 <sup>9</sup> /L		19/144
Platelet count (x 10 <sup>9</sup> /L)	687.5 (28-684)	
Platelets < 100 x 10 <sup>9</sup> /L		9/144
Circulating blasts (≥ 1%)	(0-9)	21/144
Constitutional symptoms		25/144
Palpable splenomegaly		98/137
Cytogenetics		126/144
Karyotype, normal/aberrant		90/36

n – parameterized; N – total number of patients examined; Hb – hemoglobin; WBC – white blood cell; F – female; M – male.

**Table 2**  
**Karyotype abnormalities at presentation (n = 37)**

Patient No.	Sex/age (years)	Karyotype
1	F/64	47,XX,+8[3]/48XX,+8,+10[5]/46XX[2]
2	M/77	46,XY,del(6)(p23),del(8)(q22)[20]
3	M/60	46,XY,del(13)(q12q22)[19]/46,XY[1]
4	M/68	47,XY,+mar[20]
5	M/57	46,XY,-7,+dmin[15]/46,XY[5]
6	M/62	46,XY,del(20)(q11q13)[20]
7	F/48	46,XX,rob(13;14)(q10;q10)[10]
8	F/65	47,XX,+9[1]/46,XX[19]
9	F/9	46,XX,del(13)(q12q22)[19]/46,XX[1]
10	F/64	45,XX,-18[3]/46,XX[17]
11	M/72	46,XY,del(9)(q21)[8]/46,XY[5]
12	F/62	47,XX,1qh+c,+9[5]/46,XX,1qh+c[5]
13	M/73	47,XY,+9[6]/46,XY[9]
14	F/80	46,XX,t(17;22)(q11;q13)[20]
15	M/62	47,XY,+9[3]/46,XY[13]
16	F/45	46,XX,del(9)(q22)[3]/47,XX,idem,+del(9)(q22)[17]
17	M/58	46,XY,t(15;17)(q22;q25)[15]/46,XY,add(18)(p11)[3]
18	M/63	46,XY,del(5)(q13q31)[9]/46,XY[8]
19	F/59	46,XX,del(13)(q12q22)[1]/46,XX,idem,-11,+mar[19]
20	M/70	46,XY,t(9;12)(q22;q24)[2]/46,XY[20]
21	F/70	46,XX, add(18)(p11)[18]/50-52,XX,inc[cp2]
22	M/75	46,XY,del(12)(p11p13)[3]
23	F/54	46,XX,ins(16)(q?)[2]/46,XX[8]
24	M/79	48,XY,+2mar[8]/46,XY[12]
25	M/80	47,XY,+mar[10]
26	F/70	46,XX,der(15)t(1;15)(q12;p11)[20]
27	M/75	46,XY,del(7)(q31)[12]/46,XY[8]
28	M/70	46,XY,del(7)(q32)[18]/46,XY[2]
29	F/60	46,XX,del(20)(q11q13)[20]
30	M/69	46,XY,del(20)(q11q13)[10]
31	F/66	46,XX,add(16)(q?)[11]/46,XX[3]
32	M/68	47,XY,+21[3]/46,XY[10]
33	M/73	46,XY,del(12)(p11p13)[20]
34	F/61	46,XX,inv(3)(p13q27)[3]/46,XX[7]
35	M/50	45,X,-Y[10]
36	F/70	46,XX,t(2;4)(q21;p16)[10]
37	M/76	45,X,-Yc[10]

F - female; M - male.

Partial trisomy of 1q was detected as an unbalanced translocation with chromosome 15 [der(15)t(1;15) (q12;p11)]. We registered the Robertsonian translocation, rob(13;14) (q10;q10) (Table 2, No. 7) in the karyotype of one patient. This has not been previously described in PMF. Each of the following translocations: t(17;22)(q11;q13), t(15;17) (q22;q25), t(9;12) (q22;q24), t(2;4)(q21;p16) was detected in a single patient (Table 2, Nos.14, 17, 20, and 36). Two patients had karyotypes with two unrelated pathologic clones. One of them had one clone with balanced t(15;17)(q22;q25) (Table 2, No.17) and the other had additional material to the 18p. This patient (Table 2, No. 21) had unrelated clones with add(18)(p11) and a hyperdiploid chromosome number.

#### *Impact of clinical and hematological parameters on overall survival*

The female/male ratio was 55/89 and the median age was 65 years. The median follow-up finishing in 2013 was 83 months with 49 (34%) recorded deaths. There was no difference in OS between females and males ( $p = 0.353$ ).

The stratification of patients according to clinical and hematological data, presented in Table 1, showed statistical significance for OS prediction when using Lille, Cervantes,

IPSS, DIPSS, Mayo, and Mayo PSSs for younger patients ( $\leq 60$  years) ( $p < 0.001$ ,  $p < 0.001$ ,  $p < 0.001$ ,  $p < 0.001$ ,  $p = 0.001$ , and  $p = 0.013$ , respectively) (Figure 1). The scoring of clinical and hematological parameters and stratification of risk by categories for all PSSs are presented in Table 3.

#### *Impact of the karyotype pattern on overall survival*

The impact of chromosomal aberrations on OS was estimated independently according to the CLille, CMayo, CIPSS and Refined CIPSS recommendations (Table 4). The CLille system found no statistically significant difference ( $p = 0.155$ ) in OS between the groups of patients with and without chromosome aberrations (Figure 2a). A similar result was obtained ( $p = 0.214$ ) using the CMayo system (Figure 2b).

Application of the CIPSS [8] to our data also disclosed no significant differences ( $p = 0.152$ ) in OS for patients belonging to the four distinct risk groups of this prognostic system (Figure 2c). However, the Refined CIPSS (Figure 2d) detected strong statistical significance ( $p = 0.004$ ) for a difference in OS between just two cytogenetic categories of patients (Table 3): one with a favorable karyotype (108 patients) and the other with unfavorable chromosome aberrations (18 patients).

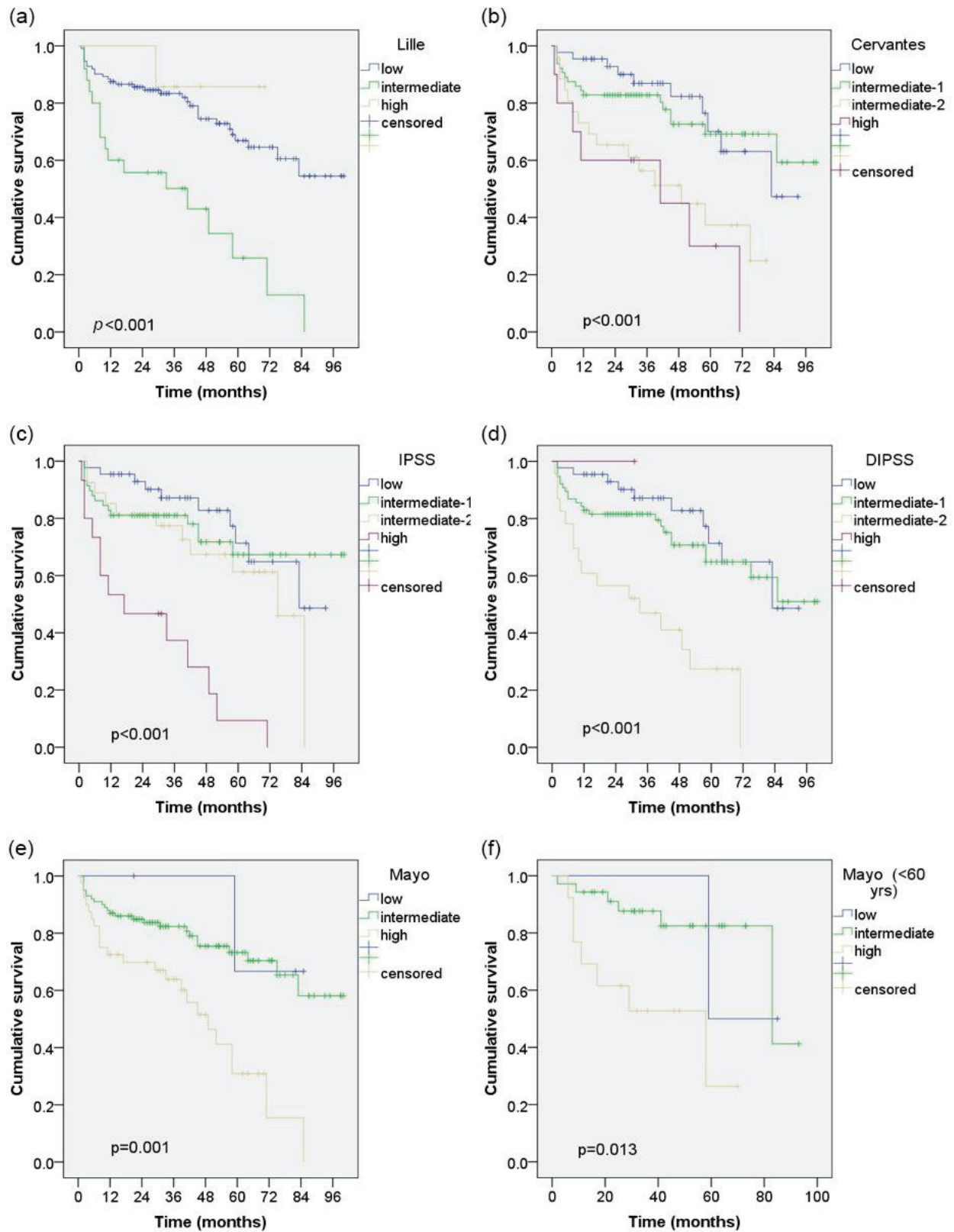
**Table 3**  
**Prognostic Scoring Systems (PSSs) for risk assessment in primary myelofibrosis patients (n = 144)**

PSSs	Prognostic parameters	Risk stratification	Risk category	Prognostic significance for surviving, <i>p</i> -values
Lille <sup>3</sup>	1. Hb (< 100 g/L)	0	low	< 0.001
	2. WBC count (< 4 or > 30 x 10 <sup>9</sup> /L)	1	intermediate	
		2	high	
Cervantes <sup>4</sup>	1. age (> 64 years)	0	low	< 0.001
	2. constitutional symptoms*	1	intermediate-1	
	3. Hb (< 100 g/L)	2	intermediate-2	
	4. blood blasts	3	high	
IPSS <sup>10</sup>	1. age (> 65 years)	0	low	< 0.001
	2. constitutional symptoms*	1	intermediate-1	
	3. Hb (< 100 g/L)	2	intermediate-2	
	4. WBC count (> 25 x 10 <sup>9</sup> /L)	$\geq 3$	high	
	5. circulating blasts ( $\geq 1\%$ )			
DIPSS <sup>11</sup>	1. age (> 65 years)	0	low	< 0.001
	2. constitutional symptoms*	1 or 2	intermediate-1	
	3. Hb (< 100 g/L)	3 or 4	intermediate-2	
	4. WBC count (> 25 x 10 <sup>9</sup> /L)	> 4	high	
	5. circulating blasts ( $\geq 1\%$ )			
Mayo <sup>12</sup>	1. Hb (< 100 g/L)	0	low	0.001
	2. WBC count (< 4 or > 30 x 10 <sup>9</sup> /L)	1	intermediate	
	3. Pt (< 100 x 10 <sup>9</sup> /L)			
	4. monocytes (> 1 x 10 <sup>9</sup> /L)			
	5. circulating blasts	$\geq 2$	high	
Mayo ( $\leq 60$ years) <sup>13</sup>	1. Hb (< 100 g/L)	0	low	0.013
	2. WBC count (< 4 or > 30 x 10 <sup>9</sup> /L)	1	intermediate	
	3. Pt (< 100 x 10 <sup>9</sup> /L)			
	4. monocytes (> 1 x 10 <sup>9</sup> /L)			

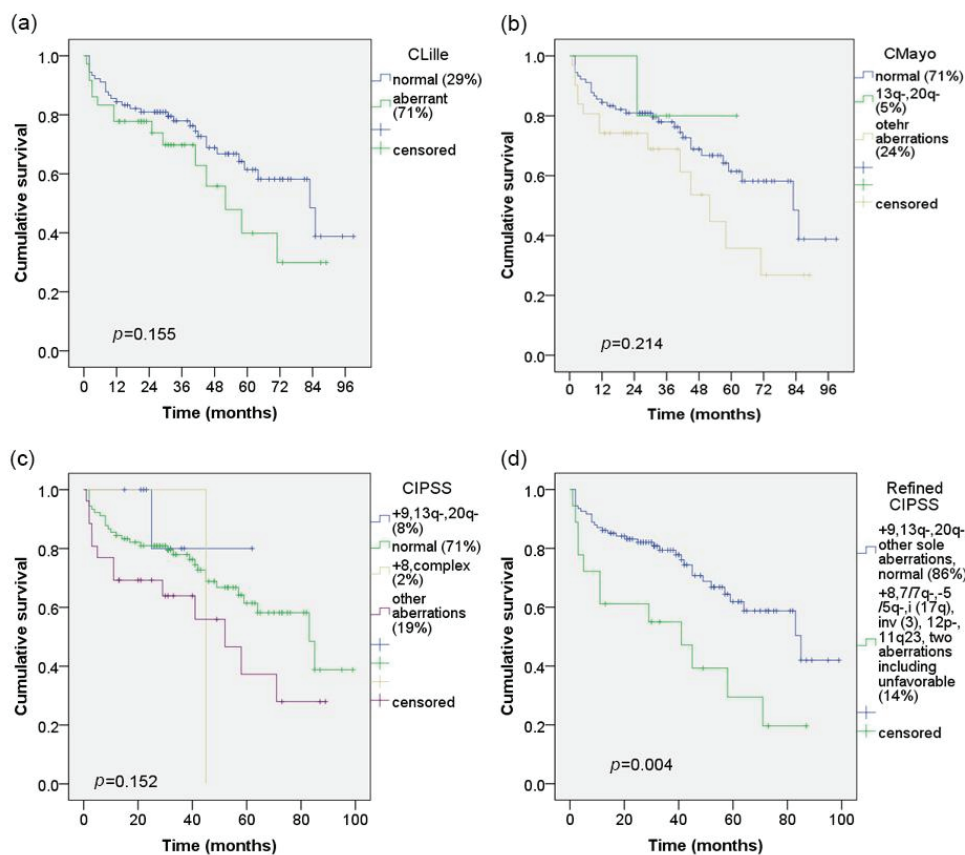
\*Constitutional symptoms included fever, night sweats or weight loss of  $\geq 10\%$  within the last 6 months.

Hb – hemoglobin; WBC – white blood cell; Pt – platelet.





**Fig. 1 – Kaplan-Meier survival curves for 144 patients with primary myelofibrosis stratified by clinical and hematological characteristics, based on the: a) Lille; b) Cervantes; c) International Prognostic Scoring System (IPSS); d) Dynamic International Prognostic Scoring System (DIPSS); e) Mayo for all patients; f) Mayo ( $\leq 60$  year-old) patients (see Table 3 for details of these prognostic systems).**



**Fig. 2 – Kaplan-Meier survival curves for 126 patients stratified by cytogenetic (C) criteria alone based on the: a) CLille; b) CMayo; c) Cytogenetic International Prognostic Scoring System (CIPSS); d) Refined CIPSS (see Table 4 for details of these prognostic systems).**

**Table 4**

**Independent Cytogenetic Prognostic Scoring Systems (CPSSs) for risk assessment (n\*=126)**

CPSSs	Karyotype	Risk category	Prognostic significance for surviving, <i>p</i> -values
CLille <sup>3</sup>	1. normal 2. aberrant	favorable unfavorable	0.155
CMayo <sup>7</sup>	1. normal 2. 13q- or 20q- 3. other aberrations	favorable unfavorable	0.214
CIPSS <sup>8</sup>	1. +9 or 13q- or 20q- 2. normal 3. other aberrations 4. +8 or complex karyotype (≥ 3 aberrations)		
Refined CIPSS <sup>9</sup>	1. sole 13q- t/dup(1q) sole 20q- sole +9 other sole aberrations two aberrations excluding unfavorable normal karyotype	favorable	0.004
	2. complex karyotype (≥ 3 aberrations) sole +8 sole -5/5q- sole -7/7q- i(17q) inv(3) 12p- 11q23 two aberrations including at least one unfavorable	unfavorable	

\*n – total number of patients with successful cytogenetics.

## Discussion

Overall survival in PMF depends on clinical, laboratory, cytogenetic and molecular characteristics of the disease. It is affected by the patient's age, physical condition and associated morbidities. In order to opt for the most favorable therapy at the time of diagnosis, it is imperative to try to pinpoint those features with the greatest impact on survival and quality of life.

Prospective studies of the prognostic impact of cytogenetic data in PMF patients are relatively rare<sup>18-20</sup>. Technical difficulties in obtaining representative aspirates from the fibrotic bone marrow make estimates of the frequency of abnormal clones difficult. In consequence, reported pathologic karyotypes in PMF range between 30% and 75%<sup>3, 5, 6, 18, 19</sup>. Among the registered aberrations in this disease, more than 90% include 20q-, 13q-, +8, +9, -Y, +21, 11q-, 12p-, partial trisomy of 1q, and rearrangements of chromosomes 5 and 7<sup>20</sup>. The aberrations 20q-, 13q- and +9 were detected in 15%-50% of cases, usually as solitary changes<sup>8, 9, 18-21</sup>.

In our study, the most common abnormalities were +9, 13q- and 20q- (28%), which is in concordance with earlier findings<sup>21</sup>. The frequency of trisomy 9 in PMF is estimated to be 5%-10%<sup>8, 20, 21</sup>, while in our group it was slightly higher – 11% (4 patients). Interstitial deletions of 13q and 20q were detected in six (17%) patients (Table 2). Molecular analyses of 13q- and 20q- identified two genes responsible for the development of myeloid disorders: the retinoblastoma gene on 13q14 and protooncogene *C-SRC1*, located distal to 20q12-q13<sup>5</sup>. Deletions of 13q- and 20q- are prominent features in PMF, suggesting that gene loss underlies the pathogenesis of the disease.

Partial trisomy of 1q is a specific aberration in PMF. Its frequency varies from only 3%<sup>3</sup> in some studies to more than 30% in others<sup>21</sup>, or is even not seen at all<sup>5</sup>. In our group, +1q was detected in a single (3%) patient [der(15)t(1;15)(q12;p11)]. It is known that rearranged 1q usually leads to gene amplification enabling proliferative advantage of the malignant clone<sup>22</sup>. This was confirmed in our case where +1q was registered in all analyzed cells.

Aberrations of chromosomes 5 and 7 are less frequent in PMF but have significant influence on the prognosis. Survival of patients with -5/5q- and -7/7q- rearrangements is inferior when compared with patients having a normal karyotype and similar to those with complex karyotypes<sup>9</sup>. We detected 5q- in one (3%) case and -7/7q- in three (8%) individuals. Those with -7 and 7q- had a lethal outcome with overall survival of 3-58 months after disease presentation, confirming published data<sup>9</sup> indicating that aberrations of -7/7q- are a poor predictive factor. While our patient with 5q- was alive 13 months after presentation, this monitoring period was too short to conclude about its impact on OS.

Two (6%) of our patients had 12p deletion. Since both individuals survived for only 2 to 3 months after diagnosis, we can conclude that del(12)(p11p13) is a very poor prognostic parameter in PMF. Patients with 12p- were classified in a high-risk category based on criteria of all tested PSSs. The most likely explanation for their short survival is an as-

sociation of poor prognostic parameters, clinical, hematological and cytogenetical.

Pericentric inversion of chromosome 3 with p13q27 breakpoints was detected in a single (3%) patient. Paracentric inversion of chromosome 3 and translocation t(3;3) with (q21q26) breakpoints represent distinct entities in acute myeloid leukemias and are recognised as a molecular marker of poor prognosis<sup>23</sup>. Our patient with pericentric inversion inv(3)(p13q27), represents the first case with this abnormality to be reported in PMF so far. At the moment of completion of the study the patient was alive (49 months) without any indication of evolution of the disease to acute leukemia.

Deletions of the long arm of chromosome 9 are a rare finding in PMF. When present, they are usually associated with other abnormalities in a complex karyotype. The most commonly rearranged bands on 9q are q21-q22, representing 46% of the total rearrangements affecting bands 9q11 to 9q33<sup>24</sup>. Interstitial deletions of 9q are relatively infrequent and have been found almost exclusively in MPN<sup>24</sup>. Terminal deletions of 9q are less common and predominantly involve the same breakpoints (q21-q22). Two (6%) of our patients had terminal 9q deletions with the above mentioned breakpoints q21 and q22. In one patient (Table 2, No. 16) we noted a clone with 9q- as a single aberration, associated with another subclone (a clone in evolution) where this aberration affected both homologues. This cytogenetic finding is rarely seen in PMF. At the moment of completion of the study, the patient was alive (73 months) without any clinical signs of disease progression. The other patient had 9q- as the sole abnormality (alive for 21 months). As the number of patients with 9q- was small, we can only speculate that 9q- aberrations do not belong to the group of poor cytogenetic parameters in PMF.

Sex chromosome aneuploidies are common numeric aberrations in hematologic diseases, including PMF. Loss of Y can be a primary or secondary event<sup>25</sup> reflecting disease evolution or a normal aging process<sup>25</sup>. Our patient with -Y, as the clonal abnormality, was alive (31 months) in 2013. We assume that clonal aberrations of the Y chromosome are not an unfavorable prognostic parameter for PMF, although the time of follow-up was short.

When compared with other studies, our results show an increased frequency of chromosomal translocations. Balanced translocations were detected in five patients (14%) (Table 2), one (3%) of whom carried the Robertsonian type. Translocations were always solitary changes in the karyotype. In one patient with t(15;17), there was another coexisting but unrelated clone with extramaterial added to the 18p (Table 2, No. 17). The mosaic karyotype of this patient represents a unique cytogenetic finding in PMF. Since the karyotype with two associated unrelated clones was present at disease presentation, it is possible that it was the first sign of leukemic transformation (after 33 months follow-up), which was not seen when analyzing other clinical and hematological parameters.

The majority of Robertsonian translocations detected in hematologic diseases are of natal origin. Welborn<sup>26</sup> analyzed the frequency of Robertsonian translocations in the karyo-

type of 5,633 patients with different hematological malignancies. Most were the rob(13;14) and rob(13;15) type and occurred equally in all types of hematological malignancies. These chromosome fusions can be induced *in vitro* in animal systems and are associated with the development of neoplasia<sup>26</sup>. In our study, rob(13;14) was a constitutional change, detected in one (3%) patient, who was alive at the moment of completion of the study and 27 months after diagnosis.

PMF is a disease that occasionally transforms into acute myeloid leukemia. It has been shown that patients with -7/7q-, -5/5q-, 12p-, aberrations of 1q, +8 and +9 transform more often (50%, 30%, 25%, 19%, 21% and 17%, respectively) than patients with 20q- or 13q- (10% and 0%, respectively)<sup>21</sup>. In our group, no case of transformation into acute leukemia was registered. Therefore, we can not conclude that aberrations carry leukemogenic potential themselves, but we can speculate about their prognostic potential. Our patients with single karyotype changes: der(15)t(1;15), del(7q), del(12p), der(16q), ins(16q), +21, as well as those with more than one chromosomal aberration: +8 with +10; del(6p) with del(8q); -7 with +dmin; del(9q) with +del(9q); +2mar; del(13q) with -11 and +mar; add(18p) with hyperdiploid karyotype, had a lethal outcome with OS of 2-71 months after disease presentation. In many of the CPSSs, aberrations +9, 13q-, and 20q- have been associated with a favorable prognosis, which was confirmed in our group of patients.

All subjects with single recurrent aberrations (+9, 13q-, 20q-), as well as those with -Y, and inv(3q), were alive at the time of the study realization (a survival period of 13-49 months after diagnosis). Similarly, all patients with balanced translocations and rob(13;14) were alive, with a survival time of 20 to 89 months. We can conclude that balanced translocations are not a poor prognostic parameter in PMF.

In this retrospective, single-center investigation covering a 7-year-period, pathologic karyotypes were found in 29% of patients at diagnosis. Reported estimates of the presence of chromosome aberrations in myeloproliferative neoplasm at presentation are ~35%<sup>6</sup>. Higher frequencies of aberrations can be expected in later phases of the disease or during the myelofibrotic transformations of essential thrombocythemia and polycythemia vera (post-essential thrombocythemia myelofibrosis and post-polycythemia vera myelofibrosis)<sup>27</sup>. Our study included only PMF patients at pres-

entation (at the beginning of the disease), which may explain the lower incidence of abnormal karyotypes compared with data in the literature.

Statistical analysis of survival based on clinical and hematologic parameters was performed for 144 patients. In all applied PSS [Lille, Cervantes, IPSS, DIPSS, Mayo, Mayo (aged  $\leq 60$  years)] the recorded survival of patients among different risk categories showed significant differences. We concluded that all of the PSSs are quite informative and equally efficient in risk estimation and prediction of PMF disease. In the group of younger patients aged  $\leq 60$  years (60 patients) we applied the Mayo PSS model.

Chromosome aberrations were treated as independent prognostic parameters and their influence on cumulative patient survival was tested by univariate analysis. More precisely, we applied various CPSSs because of their diverse definitions of benchmarks for inclusion into particular prognostic groups, i.e. the same chromosome aberrations are given disparate risk weights in different CPSSs.

Using CLille, CMayo and CIPSS, no statistical significance of karyotype profile on patient survival was documented. Normal karyotype and chromosome aberrations included in these CPSSs (+9, +8, 13q-, 20q-, complex karyotype, and all other aberrations) seemed to have no influence on patient survival, irrespective of the degree of risk. On the contrary, the Refined CIPSS showed high statistical significance regarding patient survival. However, dividing patients into two cohorts according to the karyotypic changes seems too simple for survival prediction in PMF.

## Conclusion

This study confirms that karyotype analysis remains a powerful prognostic tool and its interaction with clinical, hematological and molecular risk factors is an important driver of the disease course. It should be included in future revisions of prognostic models.

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## Incidence, in- hospital mortality and risk factors for hospital-acquired pneumonia in patients with intra-abdominal surgical procedures hospitalized in a tertiary hospital in Belgrade, Serbia: A matched case-control study

Incidenca, bolnička smrtnost i faktori rizika za nastanak bolnički stečene pneumonije kod bolesnika nakon intraabdominalnih hirurških procedura, hospitalizovanih u bolnici tercijernog nivoa zdravstvene zaštite u Beogradu, Srbija: *case-control* studija

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### Abstract

**Background/Aim.** Hospital-acquired pneumonia (HAP) in a surgical population significantly increases morbidity and mortality, prolongs hospitalization and increases total treatment costs. In the present study, we aimed to determine incidence, in-hospital mortality and risk factors (RFs) of HAP in patients with intra-abdominal surgical procedures hospitalized in a tertiary hospital in Belgrade (Serbia). **Methods.** Through regular hospital surveillance of patients who underwent intra-abdominal surgical procedures, we prospectively identified postoperative HAP during five years. In the matched case-control study, every surgical patient with HAP was compared with four control patients without HAP. In the group of patients with HAP, those who died were compared with those who survived. **Results.** Overall 1.4% of all intra-abdominal surgical patients developed HAP in the postoperative period. The incidence of HAP (per 1,000 operative procedures) was greatest in patients undergoing ex-

ploratory laparotomy (102.6), followed by small bowel surgery (36.6), and gastric surgery (22.7). Multivariate logistic regression analysis (MLRA) identified three independent risk factors (RF) associated with HAP: multiple transfusion [ $p = 0.011$ ; odds ratio (OR): 4.26; 95% confidence interval (CI): 1.59–11.33], length of hospital stay ( $p = 0.024$ ; OR: 1.02; 95%CI: 1.00–1.03) and hospitalization in the Intensive care unit (ICU) ( $p = 0.043$ ; OR: 2.83; 95%CI: 1.03–7.71). MLRA identified only surgical site infection as an independent RF associated with the poor outcome of HAP ( $p = 0.017$ ; OR: 5.929; CI95%: 1.37–25.67). **Conclusion.** The results of the present study are valuable in documenting the relations between RFs and HAP in patients undergoing intra-abdominal surgical procedures.

### Key words:

cross infection; pneumonia; digestive system surgical procedures; risk factors; incidence; mortality.

### Apstrakt

**Uvod/Cilj.** Bolnički stečena pneumonija u populaciji hirurških bolesnika značajno povećava obolevanje i smrtnost, produžava vreme bolničkog lečenja i povećava troškove lečenja. Cilj rada je bio da se odrede incidenca, bolnička smrtnost i faktori rizika (FR) za nastanak bolnički stečene

pneumonije kod bolesnika podvrgnutih intrabdominalnim hirurškim procedurama, bolnički lečenim u ustanovi tercijernog nivoa zdravstvene zaštite u Beogradu, Srbija. **Metode.** Kroz uobičajeni bolnički nadzor hirurških bolesnika nakon intraabdominalnih hirurških procedura, prospektivno smo identifikovali postoperativno nastale bolnički stečene pneumonije u periodu od pet godina. U “mečovanoj” slučaj-

kontrola studiji, svaki hirurški bolesnik sa bolnički stečenom pneumonijom je poređen sa četiri kontrolna ispitanika (bez bolnički stečene pneumonije). U grupi bolesnika sa bolnički stečenom pneumonijom, umrli su poređeni sa preživelim bolesnicima. **Rezultati.** Od svih bolesnika, 1,4% je razvilo bolnički stečenu pneumoniju u postoperativnom periodu nakon intraabdominalne hirurške procedure. Incidenca bolnički stečene pneumonije (na 1000 operativnih procedura) bila je najveća kod bolesnika koji su bili podvrgnuti eksplorativnoj laparotomiji (102,6), potom hirurgiju tankog creva (36,6) i hirurgiju želuca (22,7). Multivarijantnom logističkom regresionom analizom (MLRA) identifikovana su tri nezavisna FR udružena sa bolnički stečenom pneumonijom: multiple transfuzije [ $p = 0,011$ ; *odds ratio* (OR): 4,26; 95%

*confidence interval* (CI): 1,59–11,33), dužina bolničkog lečenja ( $p = 0,024$ ; OR: 1,02; 95% CI: 1,00–1,03) i lečenje u jedinici intenzivne nege ( $p = 0,043$ ; OR: 2,83; 95% CI: 1,03–7,71)]. MLRA je identifikovala samo infekciju hirurškog mesta kao nezavisni FR povezan sa lošijim ishodom bolnički stečene pneumonije ( $p = 0,017$ ; OR: 5,929; CI 95%: 1,37–25,67). **Zaključak.** Rezultati studije su značajni u potvrđivanju odnosa između FR i bolnički stečene pneumonije kod bolesnika podvrgnutih intraabdominalnim hirurškim procedurama.

#### Ključne reči:

**infekcija, intrahospitalna; pneumonija; hirurgija digestivnog sistema, procedure; faktori rizika; incidenca; mortalitet.**

## Introduction

Hospital-acquired pneumonia (HAP) is a very serious health problem in hospitals all over the world<sup>1–3</sup>. It is the infection of lower respiratory tract that occurs clinically two or more days after hospitalization and was not incubating at the time of hospital admission<sup>4</sup>. The reported incidence of HAP varies according to the type of population studied, ward location and length of hospital stay<sup>1,2,5</sup>. Critically ill patients admitted to intensive care units (ICUs) carry higher risk of HAP than those treated outside ICUs. Ventilator-associated pneumonia (VAP) referred to HAP develops among patients on mechanical ventilators (MV) and presents more than 48 hours after endotracheal intubation<sup>6</sup>. HAP in surgical population significantly increases morbidity and mortality, prolonging hospitalization and increasing total treatment costs<sup>7–10</sup>. Surveillance of HAP provides useful data in identifying risk factors (RF) that contribute to the development and outcome of HAP. In the present study, we aimed to determine incidence, in-hospital mortality and RFs of HAP in patients with intra-abdominal surgical procedures hospitalized in a tertiary hospital in Belgrade (Serbia).

## Methods

### Setting

The Military Medical Academy (MMA), Belgrade, Serbia, a teaching hospital of the University of Defence, is a 1200-bed tertiary healthcare center with 27 departments according to medical specialities. The Clinic for General Surgery is a 72-bed department of the MMA. The Department of Infection Control performs continuous surveillance of healthcare-associated infections (HAI), including HAP, on surgical patients of MMA.

### Study population

Through regular hospital surveillance of patients who underwent intra-abdominal surgical procedures, we prospectively identified postoperative HAP during the study period, from 1st January, 2007 to 31st December, 2011. Reviewing

the clinical chart information on patient characteristics, RFs related to health care were collected. We gathered data on the following variables: patients characteristics existing before operative procedures – gender, age, body mass index (BMI), the presence of underlying diabetes mellitus, tobacco use, preoperative infection, the American Society of Anesthesiologists (ASA) score, factors related to health care including the length of hospital stay, ICU admission, MV, central vascular catheter (CVC), histamine-2-receptor antagonists (H2RAs) use, proton-pump inhibitors (PPIs) use and preoperative antibiotic prophylaxis, red blood cell transfusion, outcome of treatment (live/dead) and characteristics of operative procedure – elective surgery, upper abdominal surgery, duration of operation, class of contamination of surgical site, drainage, duration of drainage and surgical site infection (SSI). In the case-control study, every surgical patient with HAP was compared with four control patients without HAP. Control patients were matched to the cases by age ( $\pm 5$  years), ASA score and date of surgical operation. In the group of patients with HAP those who died were compared with those who survived.

### Definition

Pneumonia is defined as “new lung infiltrates plus clinical evidence that the infiltrate is of an infectious origin, which include the new onset of fever, purulent sputum, leukocytosis, and decline in oxygenation”<sup>11</sup>. HAP was diagnosed by the consultative specialist of pulmonology based on the presence of radiographic shadowing<sup>3,4,12</sup>. SSI is defined according to the Center for Disease Control and Prevention/National Healthcare Safety Network (CDC/NHSN) surveillance definitions<sup>12</sup>. All patients were assessed before operation by anesthesiologist for the ASA score<sup>13</sup>. The National Research Council operative site classification was used by surgeon to class surgical wounds as clean, clean/contaminated, contaminated, and dirty/infected<sup>14</sup>.

Multiple transfusions is defined as more than one pack of red blood cell.

Patients with preoperative pneumonia and pneumonia that developed after postoperative respiratory failure were excluded.

No post-discharge surveillance was performed.

### Microbiological testing

The microbiological testing was performed at the MMA's Institute of Medical Microbiology. The microbiological methods were used according to the protocol for HAP included sputum or tracheal aspirate cultures and serial blood cultures.

### Statistical analysis

Incidence rate (IR) was defined as the number of HAP per 1,000 specific intra-abdominal operative procedures. The in-hospital mortality rate was defined as the number of deaths per 100 patients with HAP.

Data analyses were performed with SPSS, version 18.0 (SPSS, Inc, Chicago, IL). Results were expressed as the mean  $\pm$  standard deviation (SD) or as proportion of the total number of patients. The  $\chi^2$ -test or Fischer's exact test were used for categorical variables and relative risk, and their corresponding 95% confidence intervals (CI) were calculated. For parametric continuous variables, mean values were compared using Student's *t*-test. For nonparametric continuous variables, the Mann-Whitney *U* test was used. RFs independently associated with HAP were identified by the stepwise logistic regression analysis of variables selected by univariate analysis, with a limit for entering and removing variables at 0.05.

The informed written consent was obtained from all participants. The Ethics Committee of the MMA approved the research protocol.

## Results

### Study population

During 2007–2011 in the Clinic for General Surgery of the MMA, the surveillance of HAIs after 8,003 operative procedures was performed. In this study only patients with intra-abdominal operations were included. In the sample of 3,758 intra-abdominal operations, colorectal surgery was the most common operative procedure performed, accounting 1,524 or 40.6% (Table 1). Appendix surgery was the second most common intra-abdominal operative procedure (accounting 474 or 12.6%), followed by small bowel surgery

(accounting 464 or 12.3%), gastric surgery (accounting 441 or 11.7%) and bile duct, liver or pancreatic surgery (accounting 361 or 9.6%). Other operative procedures included exploratory laparotomy (accounting 39 or 1.0%) and spleen surgery (accounting 36 or 0.096%).

### Incidence of HAP

Overall 1.4% (51 of 3,758) of all intra-abdominal surgical patients developed HAP in the postoperative period. The incidence of HAP (per 1,000 operative procedures) was greatest in patients undergoing exploratory laparotomy (102.6 per 1,000 operative procedures), followed by small bowel surgery (36.6 per 1,000 operative procedures), gastric surgery (22.7 per 1,000 operative procedures), gallbladder surgery (9.5 per 1,000 operative procedures), colorectal surgery (8.4 per 1,000 operative procedures), bile duct, liver or pancreatic surgery (5.5 per 1,000 operative procedures) and appendix surgery (2.1 per 1,000 operative procedures). Spleen surgery was not complicated by HAP.

Forty-one surgical patients with HAP were enrolled in the case-control study. A random sample of 164 control patients matched by age ( $\pm$  5 years), ASA score and date of surgical operation were selected from a total of 3,707 potentially matched controlled subjects. For 10 patients data were incomplete, so we excluded them from the study of RF. Of 41 patients with HAP, 34 or 82.9% were treated in the ICU more than 48h and 16 or 39.0% were at some time on MV. Twelve or 29.3% patients were diagnosed with VAP.

### Risk factors for the acquisition of HAP

The patients with HAP had mean age of  $63.54 \pm 12.85$  and 61.0% were male.

Patients' characteristics, procedures during hospitalization, and characteristics related to surgical procedure performed in the case and control groups according to univariate logistic regression analysis (ULRA) are shown in Table 2. According to ULRA, the next characteristics were more frequent in cases with HAP than in controls: better outcome, longer hospitalization, hospitalization in the ICU, CVC, MV, H2RA or PPI use, multiple transfusion, preoperative antibiotic prophylaxis, elective surgery, contaminated and dirty/infected class of contamination, drainage, longer duration of drainage and SSI.

**Table 1**  
Number and percentage of specific intra-abdominal surgical procedures among all intra-abdominal surgical procedures and hospital-acquired pneumonia (HAP) rate in the study population

Surgical procedure	Number	%	HAP rate per 1,000 surgical procedures
Colorectal surgery	1,524	40.6	8.4
Appendix surgery	474	12.6	2.1
Small bowel surgery	464	12.3	36.6
Gastric surgery	441	11.7	22.7
Gallbladder surgery	419	11.1	9.5
Bile duct, liver or pancreatic surgery	361	9.6	5.5
Exploratory laparotomy	39	1.0	102.6
Spleen surgery	36	1.0	/



**Table 2**  
**Potential risk factors for acquisition and poor outcome of hospital acquired-pneumonia (HAP) in intra-abdominal surgical patients: results of univariate logistic regression analysis**

Patient characteristics	With HAP (n = 41)	Without HAP (n = 164)	<i>p</i>	Survive (n = 21)	Death (n = 20)	<i>p</i>
<b>General data</b>						
Age (years), mean ± SD	63.54 ± 12.85	64.27 ± 13.16	0.707	62.95 ± 13.07	64.15 ± 12.92	0.770
Male, n (%)	25 (61.0)	90 (54.9)	0.482	14 (66.7)	11 (55.0)	0.445
Body mass index (kg/m <sup>2</sup> ), mean ± SD	24.85 ± 3.65	25.01 ± 4.35	0.603	25.76 ± 3.54	23.90 ± 3.59	0.103
Diabetes mellitus, n (%)	6 (14.6)	10 (6.1)	0.077	3 (14.3)	3 (15.0)	0.948
Tobacco use, n (%)	14 (34.1)	44 (26.8)	0.461	6 (28.6)	8 (40.0)	0.442
ASA, n (%)			1.000			0.138
2	15 (36.6)	60(36.6)		10 (47.6)	5 (25.0)	
3	26 (63.4)	104 (63.4)		11 (52.4)	15 (75.0)	
Preoperative infection, n (%)	3 (7.3)	4 (2.4)	0.143	1 (4.8)	2 (10)	0.529
Malignancy, n (%)	15 (36.6)	85 (51.8)	0.116	8 (38.1)	7 (35.0)	0.837
Treatment outcome, n (%)	20 (48.8)	11 (6.7)	< 0.001	/	/	
<b>Characteristics related to hospitalization</b>						
Preoperative hospitalization (days), mean ± SD	12.00 ± 18.27	7.61 ± 9.51	0.725	11.52 ± 18.90	12.50 ± 18.06	0.687
Length of hospital stay (days), mean ± SD	46.63 ± 30.38	21.88 ± 19.96	< 0.001	47.19 ± 35.67	46.05 ± 24.56	0.906
Hospitalization in ICU, n (%)	34 (82.9%)	60 (36.6%)	< 0.001	15 (71.4)	19 (95.0)	0.074
Central venous catheter, n (%)	29 (70.7)	69 (42.1)	< 0.001	12 (57.1)	17 (85.0)	0.059
Mechanical ventilation, n (%)	16 (39.0)	7 (4.3)	< 0.001	5 (23.8)	11 (55.0)	0.045
H2 receptor antagonist (H2RA), n (%)	24 (58.3)	56/159 (35.2)	0.007	9 (42.9)	15 (75.0)	0.077
Proton-pump inhibitors (PPI), n (%)	13 (31.7)	29/159 (18.2)	0.059	8 (38.1)	5 (25.0)	0.572
Acid suppressive medications (H2RA or PPI), n (%)	37 (90.2)	85/159 (53.4)	< 0.001	17 (81.0)	20 (100.0)	0.107
Multiple transfusion, n (%)	38 (92.7)	62/159 (38.9)	< 0.001	18 (85.7)	20 (100.0)	0.125
<b>Characteristics related to surgical procedure</b>						
Preoperative prophylaxis, n (%)	40 (97.6)	137 (83.5)	0.046	21 (100.0)	19 (95.0)	0.488
Elective surgery, n (%)	21 (51.2)	116 (70.7)	0.019	9 (42.9)	12 (60.0)	0.275
Upper abdominal surgery, n (%)	17 (41.5)	61 (37.2)	0.746	9 (42.9)	8 (40.0)	0.853
Duration of operation (minutes), mean ± SD	121.83 ± 56.68	118.78 ± 68.49	0.473	122.38 ± 59.21	121.25 ± 55.43	0.950
Class of contamination, n (%)						0.150
clean	5 (12.2)	36 (22.0)	0.239	3 (14.3)	2 (10.0)	0.954
clean/contaminated	12 (29.3)	82 (50.0)	0.315	8 (38.1)	4 (20.0)	0.794
contaminated	6 (14.6)	17 (10.4)	0.001	2 (9.5)	4 (20.0)	0.383
dirty/infected	18 (43.9)	29 (17.7)	0.008	8 (38.1)	10 (50.0)	0.541
Drainage, n (%)	39 (95.1)	127 (77.4)	0.018	20 (95.2)	19 (95.0)	0.972
Drainage (days), mean ± SD	11.72 ± 6.35	8.93 ± 4.93	0.006	12.70 ± 7.55	10.68 ± 4.77	0.329
Surgical site infection, n (%)	16 (39.0)	14 (8.5)	< 0.001	4 (19.0)	12 (60.0)	0.010

Multivariate logistic regression analysis (MLRA) identified three independent RFs associated with HAP in surgical patients: multiple transfusion [ $p = 0.011$ ; odds ratio (OR): 4.26; 95% CI: 1.59–11.33], length of hospital stay ( $p = 0.024$ ; OR: 1.02; 95% CI: 1.00–1.03) and hospitalization in the ICU ( $p = 0.043$ ; OR: 2.83; 95%CI: 1.03–7.71).

#### *Risk factors for the poor outcome of HAP*

The mortality rate in patients with HAP in this study was 48.8% and it was significantly higher in the cases than in the control group ( $p < 0.001$ ).

Patients' characteristics, procedures during hospitalization, and characteristics depending on the surgery procedure

in the survived and patients who died according to ULRA are shown in Table 2. The patients with HAP who died had significantly greater frequency of the MV use ( $p = 0.045$ ) and SSI ( $p = 0.010$ ) than surgical patients with HAP who survived. MLRA identified only SSI as an independent RF associated with the poor outcome in surgical patients with HAP ( $p = 0.017$ ; OR: 5.929; 95% CI: 1.37–25.67).

#### *Microbiological etiology*

In 11 (26.8%) patients with HAP, microbiological etiology of the disease could be confirmed. *Pseudomonas aeruginosa* was the most frequent etiology, diagnosed in 5 cases (by blood culture in one case and by sputum or tracheal

aspirate culture in four cases of HAP). *Klebsiella* spp. was diagnosed in 4 cases (by blood culture in two cases and by sputum or tracheal aspirate culture in 2 cases of HAP). *Staphylococcus aureus* was diagnosed in 3 cases (by blood culture in one case and by sputum or tracheal aspirate culture in 2 cases of HAP). *Acinetobacter* spp. was diagnosed in 4 cases by sputum or tracheal aspirate culture.

## Discussion

HAP in patients undergoing intra-abdominal surgical procedures causes significant morbidity and mortality and prolongs hospital stays<sup>8,9</sup>. In this study we analyzed postoperative HAP in large cohort of intra-abdominal surgical patients. During the study period 51 or 1.4% of surgical patients were diagnosed with HAP in the postoperative period. The overall incidence of HAP was similar to incidence reported in the study of Delgado-Rodriguez et al.<sup>15</sup>, but lower than that reported in studies of Mohri et al.<sup>7</sup>, Thompson et al.<sup>8</sup>, and Patel et al.<sup>9</sup>. These differences could be related to differences in type of operative procedures conducted, characteristics of hospital populations studied, and surveillance methods used. In our study the incidence of HAP was greatest in the group of 39 patients undergoing exploratory laparotomy (102.6 per 1,000 operative procedures). In that population of patients, Thompson et al.<sup>8</sup> reported rate of 16.5, but their sample included 9,054 operative procedures. Ewadows et al.<sup>16</sup> estimated rate of 6.0 postoperative pneumonias per 1,000 colon surgery procedures. In our study, colorectal surgery was the most common operative procedure performed, accounting 1,524 or 40.6% of the operative procedures with HAP rate of 8.3 per 1,000 procedures.

Spleen surgery was not complicated by HAP in our patients (36 patients with splenectomy as a separate operative procedure) because we most commonly performed that procedure in younger patients after trauma or to treat underlying medical conditions such as thrombocytopenia, certain leukemia, or lymphomas.

### *Risk factors for the acquisition of HAP*

In the study of 571 elective operations for gastric cancer, Thompson et al.<sup>8</sup> found that female patients were two times more likely than male patients to develop HAP. On the other hand, Mohri et al.<sup>7</sup> found that after surgery for gastric cancer male patients had five times greater risk to acquire HAP. Our study showed that gender was not associated with HAP as well as with HAP poor outcome.

Our study identified three independent RFs for acquiring HAP: multiple transfusions, length of hospital stay and hospitalization in the ICU.

Systematic review and meta-analysis of the randomized trials conducted among hospitalized patients showed that a restrictive red blood cells (RBC) transfusion strategy compared with a liberal transfusion strategy was not associated with a reduced risk of health care-associated infection overall, although it was associated with a reduced risk of serious infection<sup>17</sup>. In a survey of 2,809 colorectal resections, trans-

fusion was the single most powerful RF for postoperative infection<sup>18</sup>. Intra- and/or postoperative blood transfusions were independent RFs for development of postoperative HAP after elective resection of gastric cancer<sup>7</sup>. Our patients with multiple transfusions were four times likely than patients without history of multiple transfusions to develop HAP ( $p = 0.011$ ; OR: 4.258; 95% CI: 1.59–11.33).

The prospective multicenter cohort study of 268 major elective abdominal surgery procedures showed that postoperative pulmonary complications, with pulmonary infection as most common (9% of all patients), had the most striking impact on length of hospital stay (median length of hospital stay was extended from 3 to 10 days)<sup>9</sup>. Also, Thomson et al.<sup>8</sup> reported that the mean length of hospital stay for intra-abdominal surgery patients who developed HAP was significantly greater compared with patients who did not develop HAP ( $17.10 \pm 18.66$  vs.  $6.07 \pm 5.37$  days;  $p < 0.001$ ). In our study, length of hospital stay in patients with HAP was  $46.63 \pm 30.38$  days, and in patients without HAP it was  $21.88 \pm 19.96$  days ( $p = 0.024$ ; OR: 1.02; 95% CI: 1.00–1.03). Prolonged hospitalization in our patients is explain by the fact that the majority of our patients were primarily hospitalized at the Clinic for Gastroenterology of the MMA because of implementation of preoperative diagnostic procedures, which was then followed by the hospitalization at the Clinic of Abdominal Surgery where surgery was performed.

HAP is a frequent and severe infection in the ICU, with the highest morbidity and mortality<sup>19</sup>. Alp et al.<sup>20</sup> showed that the rate of HAP, in patients in the ICU was much higher in medical than in surgical patients (11.7% vs 5.8%). Also, they showed that MV was more frequently used in medical than surgical patients ( $p < 0.01$ ). In our study of intra-abdominal surgical patients with HAP, 34 or 82.9% were treated in the ICU more than 48h and 16 or 39.0% were at some time on MV. In the study of the ICU treated patients, Karhu et al.<sup>2</sup> reported that 80% of HAP patients needed MV. Our study showed that the ICU and MV were associated with the acquisition of HAP, but MV did not retain significance as an independent RF in MLRA.

In a large, hospital-based pharmacoepidemiologic cohort study, Herzig et al.<sup>21</sup> found that acid-suppressive medication use was associated with 30% increased odds of HAP. In subset analyses, the risk for HAP was significantly increased with PPI, but not with histamine H2RA. Our patients with HAP received acid suppressive medications (H2RA or PPI) more frequently than patients in the control group (90.4% vs. 53.4%, respectively), but according to ULRA only H2RAs significantly increased the risk of HAP ( $p = 0.007$ ), without being significant independent RF.

### *Risk factors for the poor outcome of HAP*

The combination of hospital-acquired pneumonia and ventilator-associated pneumonia constitutes the most common cause of death among all hospital-acquired infections, with mortality rates of up to 33%<sup>22</sup>. In surgical population, mortality from postoperative HAP ranges from 10.7%<sup>8</sup> to 45%<sup>23</sup>. The prognosis in patients with HAP depends primar-

ily on host defenses, existing comorbidities and initial empiric therapy. The mortality rate in patients with HAP in our study was 48.8% and it was significantly higher in the case than in the control group ( $p < 0.001$ ). In our patients HAP was not the primary cause of death but it was mentioned in the clinical chart information. We identified SSI as an independent RF for the poor outcome of HAP. Some previous study of the relationship between hospital-acquired infection and in-hospital mortality in surgical patients showed that the association of SSI and either a respiratory tract infection or a bloodstream infection also significantly increased the risk of in-hospital mortality<sup>24</sup>. The longitudinal study based on prevalence data from a large emergency and referral teaching hospital in Norway, found that hospital-acquired bloodstream infection, hospital-acquired lower respiratory tract infections or more than one simultaneous hospital-acquired infection were independently and strongly associated with increased mortality 30 days and one year after inclusion in the study<sup>25</sup>.

#### Microbiological etiology

After systematic review Jones concluded that top six most prevalent pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* species, *E. coli*, *Acinetobacter* species, and *Enterobacter* species) consistently cause 80% of all HAP or VAP episodes, in contrast to only 3.7–7.3% by *S. pneumoniae* and *Haemophilus* spp.<sup>26</sup>. As reported in the study of Sopena et al.<sup>5</sup>, the etiology of HAP was known in less than one-third of our patients because of the inability to perform the invasive diagnostic procedure in most of cases. In our study identification of the causative agent was possible in only 11 or 26.8% patients with HAP. *Pseudomonas aeruginosa* was the most frequent causative agent of HAP in our study.

#### Limitation and strength of the study

Limitation of the study is the possibility of presence of confounding variables that were not examined. Although confounding variables were chosen after an exhaustive search of the literature, the potential for oversight and exclusion does exist. We did not include some parameters, namely existing of chronic obstructive pulmonary diseases and other chronic lung diseases, alcohol use, appropriate empiric treatment and analyzing these factors could have enhanced the relevance of our results. Furthermore, we did not evaluate the HAP cases and the controls in relation to the age and ASA score, because HAP cases and controls were matched according to them. Besides, this was the single centre study and the number of patients with HAP was relatively small. Finally, our mortality outcome was limited to in-hospital mortality rate. However, it is unlikely that other measures of mortality, such as 30-day mortality, would give more precise RF for the poor outcome of HAP.

The strength of our study is that it could be generalized to surgical patients with intra-abdominal surgical procedures.

#### Conclusion

During the study period, 1.4% of intra-abdominal surgical patients were diagnosed with HAP in the postoperative period. We identified three independent risk factors for acquiring HAP: multiple transfusions, length of hospital stay and hospitalization in the Intensive care unit. Also, we identified SSI as an independent RF for the poor outcome of HAP. The results of the present study are valuable in documenting the relations between RFs and HAP in patients undergoing intra-abdominal surgical procedures.

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## Parameters of radical resection in laparoscopic and open colon and rectal cancer surgery

### Parametri radikalnosti kod laparoskopske i otvorene hirurgije karcinoma kolona i rektuma

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#### Abstract

**Background/Aim.** In colon and rectal cancer surgery, resection is considered radical when circumferential, proximal and distal resection margins are without the presence of tumor cells. Concept of total mesorectal excision in rectal surgery involves complete removal of the tumor with mesorectal fascia which surrounds lymph nodes, lymphatics and blood vessels. The aim of this study was to determine whether laparoscopic approach provides all parameters of oncological radicality as open surgery of colorectal cancer. **Methods.** The study included 122 patients with carcinoma of colon and rectum, divided into two equal groups: patients operated on by laparoscopic and those operated on by open approach. In colon surgery we analyzed proximal and distal resection margins, and the number of removed lymph nodes, and in rectal surgery: proximal, distal and circumferential resection margins, and the number of removed lymph nodes. **Results.** Both groups were comparable in age, sex, American Society of Anesthesiologists (ASA) score, tumor localization, tumor size, and type of surgical operation performed. According to localization of the tumor, the most commonly performed operation was anterior resection of

the rectum (60.7% vs. 59%). There was no case of the tumor involvement of the distal margin. Average proximal distance from the tumor on the fixed specimen was 100 vs. 120 mm with statistical significance ( $p < 0.001$ ). Distal margins were not significantly different, 40 mm in both groups ( $p = 0.143$ ). In two cases we had circumferential resection margin (CRM) of 1 mm (7.7%) in the laparoscopic group, and in three cases operated conventionally CRM was 1 mm (8.8%). The average number of removed lymph nodes was 15 vs. 16, respectively. Length of hospital stay for patients assigned to the laparoscopic surgery was significantly shorter than for patients operated on by the open approach. Concerning postoperative complications, no significant difference was found between groups. The overall postoperative morbidity was 18% vs. 21.3%, respectively. **Conclusion.** With laparoscopic approach it is possible to provide all parameters of oncological radicality similarly to the open surgery of colorectal cancer.

#### Key words:

colon neoplasms; laparoscopy; laparotomy; rectal neoplasms; surgical procedures, operative; treatment outcome.

#### Apstrakt

**Uvod/Cilj.** Resekcija kod karcinoma kolona i rektuma smatra se radikalnom kada su proksimalna, distalna i radikalna linija resekcije bez prisustva tumorskih ćelija. Koncept totalne mezorektalne ekscizije podrazumeva kompletno uklanjanje tumora unutar fascijalnog omotača zajedno sa limfnim čvorovima, limfaticima i krvnim sudovima duž kojih tumor može da se širi. Cilj rada bio je da se utvrdi da li se laparoskopskim pristupom obezbeđuju svi parametri onkološke radikalnosti kao i u otvorenoj hirurgiji karcinoma

kolona i rektuma. **Metode.** Studijom su bila obuhvaćena 122 bolesnika sa karcinomom kolona i rektuma svrstana u dve jednake grupe - bolesnici operisani laparoskopskim i oni operisani otvorenim pristupom. U hirurgiji kolona analizirali smo proksimalnu i distalnu resekcionu liniju i broj uklonjenih limfnih žlezda, a u hirurgiji rektuma proksimalnu, distalnu i cirkumferentnu resekcionu liniju kao i broj uklonjenih limfnih žlezda. **Rezultati.** Obe grupe su bile jednake u odnosu na životno doba, pol, rezultat klasifikacije Američkog društva anesteziologa, lokalizaciju, veličinu tumora i vrstu operativnog zahvata. Prema lokalizaciji tumora, naj-

češće izvedena operacija bila je prednja resekcija rektuma (60,7% u odnosu na 59%). Nije bilo slučajeva sa pozitivnom distalnom linijom resekcije. Prosečno rastojanje tumora od proksimalne linije resekcije na fiksiranom preparatu bilo je 100 mm u odnosu na 120 mm, sa statistički značajnom razlikom ( $p < 0,001$ ). Udaljenost od distalne linije resekcije bila je bez razlike, 40 mm u obe grupe ( $p = 0,143$ ). Nađana je pozitivna cirkumferentna resekciona linija kod dva bolesnika u laparoskopskoj grupi (7,7%), a kod tri bolesnika u grupi operisanih otvorenom metodom (8,8%). Prosečan broj uklonjenih limfnih čvorova iznosio je 15 u laparoskopskoj grupi i 16 u grupi operisanoj otvorenim pristupom. Dužina boravka u bolnici kod bolesnika operisanih laparoskop-

skim pristupom bila je kraća, što je bilo visoko statistički značajno. U pogledu postoperativnih komplikacija, nije utvrđena značajna razlika između grupa. Ukupni postoperativni morbiditet iznosio je 18% prema 21,3%. **Zaključak.** Laparoskopskim pristupom je moguće obezbediti sve parametre onkološke radikalnosti jednako kao i u otvorenoj hirurgiji karcinoma kolona i rektuma.

#### **Ključne reči:**

**kolon, neoplazme; laparoskopija; laparotomija; neoplazme, rektum; hirurgija, operativna, procedure; lečenje, ishod.**

## **Introduction**

Traditionally, surgical management of colon cancer entails removal of the tumor together with potentially metastatic nodes. Standard oncological principle involves removal of central lymph nodes with a negative line of resection.

Recently, concept of complete mesocolic excision (CME)<sup>1</sup> has been adopted as a more radical approach to the treatment of colon carcinoma. Dissection along embryonic layers (avoiding an incomplete excision, i.e. damaged mesentery) ensures complete removal of lymph nodes and nervous tissue found in the drainage area of tumor. High vascular tie, removal of specimen with intact fascia and peritoneum, and adequate distal and proximal resection margins with maximum number of lymph nodes show better results in terms of local recurrence and survival in comparison to standard colectomy<sup>2,3</sup>. Patients at the stage II of the disease can also have a better prognosis with wider excision of mesentery with as many negative lymph nodes as possible<sup>4</sup>. Implementation of this technique, ensuring specimen with intact mesocolic fascia, is associated with 15% better five-year survival compared to specimens where there are defects in the mesocolon. The percentage of survival is even more pronounced in the stage III, up to 27%<sup>5</sup>.

By the seventies of the last century, surgical technique of blind dissection along the presacral fascia was associated with frequent injuries of presacral venous plexus and conical narrowing of specimen. Lateral excision was generally insufficient with a high percentage of local recurrence, until the introduction concept of total mesorectal excision (TME) 1982<sup>6</sup>.

The TME concept involves complete removal of the tumor with mesorectal fascia with lymph nodes, lymphatics and blood vessels of the rectum. Resection is considered radical when circumferential, proximal and distal resection margins are without presence of tumor cells. If circumferential resection margin (CRM) is positive, local recurrence is increased three to four times<sup>7</sup>. By adopting the TME technique, rate of local recurrence was decreased to 4%<sup>8</sup>. The concept of TME remains the gold standard in rectal cancer surgery.

From oncological aspect, since the first recorded colon resection<sup>9</sup>, laparoscopic colorectal surgery is still controver-

sial. Several randomized multicenter studies have shown that there is no significant difference between laparoscopic and open colon surgery when discussing the parameters of radicality: proximal, distal and radial resection margins, and the number of removed lymph nodes<sup>10,11</sup>.

In laparoscopic surgery for rectal cancer, results of the first randomized trials are promising. The CRM status, quality of total mesorectal excision and the number of removed lymph nodes did not show a significant difference between open and laparoscopic surgery<sup>12,13</sup>. However, long-term results are still missing in order to definitively determine routine use of laparoscopic surgery in this group of patients.

Analyzing the parameters of radical resection, the aim of this study was to determine whether laparoscopic surgery meets oncological principles as open surgery of colon and rectum carcinoma.

## **Methods**

The study conducted at the Clinical Hospital Center „Zvezdara“, Belgrade included 122 patients with adenocarcinoma of the cecum, ascending, descending, sigmoid colon and adenocarcinoma of the rectum. Among all of the patients, 61 were operated by laparoscopic and 61 by open approach.

Mechanical preparation of intestine, antibiotic prophylaxis and prophylaxis of deep venous thrombosis were carried out by standard procedures, regardless of the applied method of operative treatment (laparoscopic or open surgery).

Inclusion criteria were: patients without distant metastases with solitary adenocarcinoma of the cecum, ascending, descending and sigmoid colon and patients with solitary adenocarcinoma of the rectum.

Exclusion criteria included: T4 tumors of the colon and rectum, tumors that are not adenocarcinomas, patients with clinical presentation of acute intestinal obstruction, preoperatively established metastases, absolute contraindication for general anesthesia or prolonged pneumoperitoneum.

In the colon surgery we analyzed proximal and distal resection margins, and the number of removed lymph nodes, while in the rectal surgery we analyzed: proximal, distal and circumferential resection margins, and the number of removed lymph nodes.

The following surgical procedures were performed: proximal ligation of a blood vessel that supplies the tumor or multiple blood vessels if the arterial distribution is such that tumor is at an equal distance between two blood vessels, appropriate proximal and distal resection lines (minimum 5-10 cm), and adequate lymphadenectomy<sup>14</sup>; partial mesorectal excision for tumors of the upper third of rectum (distal line of resection at least 5 cm); TME was performed for tumors of middle and lower third of rectum (distal line of resection 1–2 cm); ligation of the inferior mesenteric artery at the origin (1 cm from the aorta or after the separation of the left colic artery<sup>15</sup>).

Microscopic analyses of specimens were determined by standard hematoxylin-eosin method by a pathologist at the University Clinical Center Zvezdara.

Results were presented as count (%), means  $\pm$  standard deviation or median (25th-75th percentile) depending on data type and distribution. Groups were compared using parametric (*t*-test) and nonparametric ( $\chi^2$ , Mann-Whitney *U* test, Fisher's exact test) tests. All *p* values less than 0.05 were considered significant. All data were analyzed using SPSS 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

## Results

A total of 122 patients entered the study. The average age of patients was  $67.3 \pm 10.3$  years. The youngest patient was 27 years old and the oldest one was 87 years old. Among all the patients, 77 (63.1%) were male and 45 (36.9%) female. Patients were divided into two equal groups, 61 patients in each (Table 1).

Both groups were comparable in age, sex, American Society of Anesthesiologists (ASA) score, tumor localization, tumor size, and type of surgical operation performed.

The ASA status of patients in both groups did not differ. In both groups, most frequent localization of the tumor was the rectum (39.3% vs. 52.5%). Only one patient (open technique) received neoadjuvant therapy (Table 1).

In both groups there was no statistically significant difference in relation to the type of surgical procedure (Table 2). According to the localization of the tumor, the most commonly performed operation was anterior resection of the rectum (60.7% vs. 59%). There was no case of the tumor involvement of the distal margin. Average proximal distance from the tumor on the fixed specimen was 100 vs. 120 mm with statistical significance ( $p < 0.001$ ). Distal margins were not significantly different (40 mm in both groups) ( $p = 0.143$ ). We had in two cases CRM of 1 mm (7.7%) in the laparoscopic group, and in three cases operated on conventionally, CRM was 1 mm (8.8%). The average number of removed lymph nodes was 15 vs. 16. The tumor size, stage distribution, and histological typing were similar in both groups. According to the TNM classification, in the laparoscopic group, 15 (24.6%) patients was in the stage I, 20 (32.8%) in the stage II, and 26 (42.6%) in the stage III. In the open surgical group 7 (11.5%) patients were in the stage I, 20 (32.8%) in the stage II, and 34 (55.7%) in the stage III.

We had two conversions (3.27%) to laparotomy due to technical difficulties (Table 3). Some patients already had previous abdominal operations (13.1% of patients operated laparoscopically, and 9.8% with open surgery). Length of hospital stay for patients assigned to laparoscopic surgery was shorter than for patients of the open surgery group. This difference was highly statistically significant. The mean postoperative stay was 9 (range 5–58) vs. 12 (range 5–37) days, respectively. The prolonged hospital stay of 58 days was observed in one patient with high stoma output syndrome with consequent renal insufficiency after laparoscopic low rectal resection.

**Table 1**  
Preoperative characteristics of patients with either laparoscopic or open approach

Parameter	Procedure		<i>p</i>
	laparoscopic	open	
Age (years), mean $\pm$ SD	68.0 $\pm$ 10.7	66.1 $\pm$ 9.9	0.448 <sup>a</sup>
Sex (man), n (%)	41 (67.2)	36 (59.0)	0.348 <sup>b</sup>
ASA, n (%)			
1	6 (9.8)	0	
2	28 (45.9)	31 (50.8)	0.267 <sup>c</sup>
3	25 (41.0)	27 (44.3)	
4	2 (3.3)	3 (4.9)	
Localization of carcinoma, n (%)			
right colon	17 (27.9)	16 (26.2)	0.265 <sup>b</sup>
left colon	20 (32.8)	13 (21.3)	
rectum	24 (39.3)	32 (52.5)	
Neoadjuvant therapy, n	0	1	–
Previous operations, n (%)	8 (13.1)	6 (9.8)	0.570 <sup>b</sup>

ASA - American Society of Anesthesiologists; SD – standard deviation.

<sup>a</sup>*t* test; <sup>b</sup>Pearson  $\chi^2$  test; <sup>c</sup>Mann-Whitney *U* test.

Table 2

## Operative characteristics of patients

Parameter	Procedure		p
	laparoscopic	open	
Operative procedure, n (%)			
subtotal colectomy	1 (1.6)	2 (3.3)	
right hemicolectomy	16 (26.2)	16 (26.2)	
left hemicolectomy	3 (4.9)	4 (6.6)	1.000 <sup>b</sup>
resection of sigmoid colon	4 (6.6)	3 (4.9)	
rectal resection	37 (60.7)	36 (59.0)	
Anastomosis, n (%)			
stapler	39 (63.9)	31 (50.8)	0.290 <sup>b</sup>
suture	17 (27.9)	21 (34.4)	
Tumor dimension (mm), mean ± SD	42.9 ± 16.6	45.6 ± 15.0	0.350 <sup>a</sup>
Proximal resection line (mm), average (min-max)	100 (80–130)	120 (100–170)	< 0.001 <sup>c</sup>
Distal resection line (mm), average (min-max)	40 (30–65)	40 (30–120)	0.143 <sup>c</sup>
<sup>1</sup> CRM (mm), average (min-max)	17 (6–20)	12 (5–20)	0.697 <sup>d</sup>
Positive, n (%)	2 (7.7)	3 (8.8)	1.000 <sup>b</sup>
Number of harvested lymph nodes, average (min-max)	15 (2–65)	16 (3–42)	0.756 <sup>d</sup>
Tumor differentiation, n (%)			
G1	5 (8.2)	1 (1.6)	
G2	52 (85.2)	53 (86.9)	0.092 <sup>c</sup>
G3	4 (6.6)	7 (11.5)	
pT stage, n (%)			
I	7 (11.5)	1 (1.6)	
II	7 (11.5)	7 (11.5)	0.009 <sup>c</sup>
III	41 (67.2)	47 (77.0)	
IV	2 (3.3)	6 (9.8)	
pN stage, n (%)			
0	35 (57.4)	27 (44.3)	
1	15 (24.6)	22 (36.1)	0.232 <sup>c</sup>
2	11 (18.0)	12 (19.7)	
pM 1 stage, n	0	1	-
Stage of disease, n (%)			
I	15 (24.6)	7 (11.5)	
II	20 (32.8)	20 (32.8)	0.070 <sup>c</sup>
III	26 (42.6)	34 (55.7)	

<sup>1</sup>CRM – circumferential resection margia; 1 mm was considered as positive; SD – standard deviation;

pT – primary tumor; pN – regional lymph nodes; pM – distant metastasis; min – minimum; max – maximum.

<sup>a</sup>t-test; <sup>b</sup>Pearson  $\chi^2$  test/Fisher's exact test; <sup>c</sup>Mann-Whitney U test.

Table 3

## Postoperative characteristics of patients

Parameter	Procedure		p
	laparoscopic	open	
Operative complications, n	0	1	
Conversion to laparotomy, n	2	0	
Length of hospital stay (days), median (min-max)	9 (5–58)	12 (5–37)	0.002 <sup>d</sup>
Postoperative complications, n (%)			
no	50 (82)	48 (78.7)	
surgical	7 (11.5)	7 (11.5)	0.802 <sup>b</sup>
non-surgical	4 (6.6)	6 (9.8)	
Reoperation, n (%)	4 (6.6)	2 (3.3)	0.680 <sup>b</sup>
Death, n	1	1	
Rehospitalization, n (%)	2 (3.3%)	0	0.496 <sup>b</sup>
Complications, n			
significant hemorrhage	3	0	0.244 <sup>e</sup>
ileus	1	1	1.000 <sup>e</sup>
anastomotic dehiscence	1	3	0.619 <sup>e</sup>
wound infection	2	3	1.000 <sup>e</sup>
clostridial colitis	1	2	1.000 <sup>e</sup>
cardiac insufficiency	1	1	1.000 <sup>e</sup>
renal insufficiency	1	0	0.496 <sup>e</sup>
pulmonary insufficiency	0	1	1.000 <sup>e</sup>

Among all of the patients, 61 were operated by laparoscopic and 61 by open approach.



Concerning postoperative complications, no significant difference was found between groups. The overall postoperative morbidity was 18% vs. 21.3%. Although reoperation was necessary in both groups of patients (6.6% vs. 3.3%), there was no statistically significant difference between groups ( $p = 0.680$ ). Three patients operated by laparoscopy had postoperative bleeding and all of them were reoperated. There was one case of prolonged postoperative ileus in the laparoscopic group, solved surgically. In the open surgery group we reoperated two patients due to medically unresolved ileus and anastomotic leak. There were two infections of the mini-laparotomy incision site vs. three wound infections in the open surgery group. Rehospitalization was necessary in two patients after laparoscopic surgery, which was close to statistical significance ( $p = 0.496$ ). The cause of rehospitalization was dehydration.

Overall anastomotic leakage rate was low in both groups, 1.64% vs. 4.91%. There was one case of anastomotic leakage in a patient with laparoscopic anterior rectal resection, and in three patients in the open surgery group (one with rectal resection and two with colon resection).

There was one death recorded in the laparoscopic group, due to cardiopulmonary complications after reoperation, and one in the open surgery group in case of respiratory failure.

## Discussion

Open colectomy has been the standard treatment for colon cancer patients in the past 100 years. From perineal excision and first successful radical surgical treatment of rectal cancer reported by Miles in 1908 we are witnessing the great progress of surgery during the last century<sup>16</sup>. Over the last two decades, laparoscopic approach changed surgical treatments for colorectal cancers. At beginning, there was a lot of controversies in laparoscopic surgery for colorectal cancer. Several reports of early wound recurrence raised concerns about validity of this approach<sup>17, 18</sup>. Undoubtedly, less pain, shorter recovery, and quicker return to baseline functions are well known advantages of laparoscopic approach but concerns about oncological adequacy are still present. Recently, several randomized multicenter studies have shown that there is no significant difference between laparoscopic and open colorectal surgery regarding to parameters of radicality: proximal, distal and radial lines of resection, and number of removed lymph nodes<sup>10, 11</sup>.

In our study, no differences were recorded between laparoscopic and open surgery with respect to tumor and nodal status, and short-term endpoints.

Concerning histopathological results of specimens, we can conclude that laparoscopic approach provides cancer clearance which is comparable to that of conventional surgery. In the present series there were no cases of the tumor involvement of the proximal or distal margins. Korolija et al.<sup>19</sup> showed a statistically significant difference in the average distal margin in a meta-analysis of 16 comparative series, 46 mm by the laparoscopic approach and 53 mm with the open approach. Schwenk et al.<sup>20</sup> found no difference in resection margins between two groups, what we confirmed in our study. For patients undergoing laparoscopic anterior

resection for rectal cancer, the positivity rates of surgical CRMs were also similar between the two treatment groups.

Guillou et al.<sup>11</sup> noted positive CRMs in 12% of patients after laparoscopic anterior resection for cancer whereas only 6% of patients after open surgery had positive margins. Meta-analysis of Arezzo et al.<sup>21</sup> showed us that randomized-controlled trials reported a positive circumferential margin in 7.9 % of patients who underwent laparoscopic and 6.9 % of those undergoing open surgery. Data from the non-randomized-controlled trials reported a positive circumferential margin in 8.0 % vs. 12.7 % of patients. Using 1 mm margin as positive CRM, our results (7.7% vs. 8.8%) were without statistical differences between groups and comparable with so far published results in the literature<sup>22-24</sup>.

On average, we removed fifteen lymph nodes per patient, with no significant differences between two groups, and it was also more than recommended to ensure radical resection<sup>25</sup>. Two prospective randomized trials from Europe (Barcelona, Spain<sup>26</sup>, and Colon Cancer Laparoscopic or Open Resection (COLOR) trial<sup>27</sup>) demonstrated an equivalent number of lymph nodes retrieved from both groups.

Early randomized controlled trials for colorectal cancer published high conversion rates, up to 29%, with participating surgeons who already had 20 laparoscopic procedures<sup>27, 28</sup>.

Meta-analysis by Noel et al.<sup>29</sup> reviewing all clinical studies published between 1994, and 2005, confirmed better conversion rates for malignancy, estimated at 14.8%. In our series, only two patients (3.27%) we had to convert to open surgery. The reason for such a low rate of conversion could be that all laparoscopic operations were performed by a surgeon who already has experience in the field of colorectal surgery and laparoscopy.

Although Stevenson et al.<sup>30</sup> have found no differences between the length of hospital stay, other studies<sup>13, 31-33</sup> have shown shorter hospital stay in laparoscopic surgeries of this type, which is confirmed by our results with high statistical significance ( $p = 0.002$ ). Median hospital stays (9 vs. 12 days) in our study coincides with the results of Braga et al.<sup>34</sup> study.

Overall postoperative morbidity was 18% vs. 21.3% that is slightly lower than in published series of the Clinical Outcomes of Surgical Therapy (Cost) study<sup>28</sup> (21% vs. 20%) and lower than in the Medical Research Council Conventional versus Laparoscopic-Assisted Surgery in Colorectal Cancer (MRC CLASICC) trial<sup>11</sup> (29% vs. 31%). Whereas overall anastomotic leakage rate was low, it was higher in the open group, 4.91% vs. 1.64%. For rectal resections, rate of leakage (2.7%) was the same in both groups, which was lower than Guillou et al.<sup>11</sup> reported. For colon resections in laparoscopic group we did not have clinical manifestations of anastomotic dehiscence.

In-hospital mortality also was without statistical difference in both groups (1.63%), which confirms the short-term clinical safety of the laparoscopic approach in our institution.

## Conclusion

Based on our results, we can conclude that laparoscopic approach in the treatment of colorectal cancer provides valid oncological resection as the open surgery does.

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## Is adjunctive perampanel beneficial for Lafora disease?

### Da li je primena perampanela korisna u lečenju Laforine bolesti?

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#### Abstract

**Background/Aim.** Lafora disease (LD) is progressive myoclonus epilepsy, characterized by intractable myoclonus and seizures, inevitable neurological deterioration, brutal cognitive decline and poor prognosis. The treatment still remains purely symptomatic. Recently, two single-case studies and one case series study reported the favourable effects of perampanel in LD. Our study aimed to test the benefits reported in three separate case studies. **Methods.** We performed an open label, prospective study of 4 patients aged between 22 and 34 years with mutation in NHLRC1 (EPM2B) gene, treated with perampanel (6–8 mg/day) as add-on therapy. Follow-up period comprised 14–26 months. Seizure frequency, myoclonus, functional disability and cognitive performance were analysed. **Results.** In 3 patients, both, seizures and myoclonus, showed remarkable improvement after the drug introduction (> 50% reduction). No significant effect was seen in one case. The functional and cognitive impairment maintained at the same level, though all patients were at the later stage of the disease. Psychiatric side effects were dose related. **Conclusion.** Our study supports the rare, previously reported observations that perampanel is beneficial in treating LD patients.

#### Key words:

lafora disease; diagnosis; anticolvulsants; perampanel; treatment outcome.

#### Apstrakt

**Uvod/Cilj.** Laforina bolest (LB) je progresivna mioklonička epilepsija koja se odlikuje tvrdokornim mioklonusom i napadima, neumoljivim neurološkim i brutalnim kognitivnim propadanjem i lošom prognozom. Terapija je, za sada, isključivo simptomatska. Nedavno su dve studije pojedinačnih slučajeva i jedna sa prikazom serije bolesnika pokazale povoljan efekat perampanela na LB. Cilj rada je bio da se istraži povoljan uticaj primene perampanela u terapiji LB, kako je to prikazano u malom broju studija u dostupnoj literaturi. **Metode.** Sprovedena je otvorena, prospektivna studija na 4 bolesnika, uzrasta 22–34 godine, sa mutacijom u NHLRC1 (EPM2B) genu, koji su lečeni perampanelom 6–8 mg/dnevno, kao dodatnom terapijom. Period praćenja je bio 14–26 meseci. Procenjavani su učestalost napada, mioklonus, funkcionalna onesposobljenost i kognitivno funkcionisanje. **Rezultati.** Nakon uvođenja terapije postignuta je značajno bolja kontrola napada i došlo je do smanjenja (> 50%) mioklonusa kod 3 bolesnika. Kod jednog bolesnika nije zapažen povoljan terapijski odgovor. Funkcionalno i kognitivno poboljšanje nije uočeno, iako su svi bolesnici bili u kasnijim stadijumima bolesti. Psihijatrijska neželjena dejstva su bila dozna zavisna. **Zaključak.** Našom studijom podržana su retka iskustva da je perampanel koristan u lečenju bolesnika sa LB.

#### Ključne reči:

laforina bolest; dijagnoza; antiepileptici; perampanel; lečenje, ishod.

#### Introduction

Lafora disease (LD) is very rare, an autosomal recessive, progressive metabolic disorder characterized by intractable myoclonus and seizures, inevitable neurological deterioration, brutal cognitive decline, unfavourable clinical course, and poor prognosis<sup>1</sup>.

LD in majority of patients is caused by mutations in either the EPM2A or EPM2B gene, which encode the laforin

glycogen phosphatase and the malin ubiquitin E3 ligase, respectively. These proteins have important role in glycogen metabolism due to not yet fully understood pathophysiological mode of action. Hallmark of pathological examination is accumulation of polyglucoson inclusion bodies, called Lafora bodies, in the cytoplasm of various cells, the most striking in neuronal cell bodies and dendrites<sup>2,3</sup>.

Clinical presentation appears during late childhood or adolescence (usually between 8 and 18 years of age), with an in-

sidious appearance of headaches, learning disability, focal occipital seizures, pharmacoresistant generalized tonic-clonic seizures (GTCS) and intractable myoclonus. Myoclonus can be fragmentary, symmetric, or massive and could be the primary reason for early wheelchair dependency. During the course of the disease, severe neurological and cognitive deterioration, dementia, intractable epilepsy and vegetative state led to early death, usually within the first decade from the disease onset.

The treatment of patients with LD still remains purely symptomatic, with antiepileptic and antimyoclonic drugs. Usually, they continue to experience disabling seizures and myoclonus. Two recent single-case studies<sup>4, 5</sup> and one case series study<sup>6</sup> reported the beneficial effects of the relatively new antiepileptic drug (AED), selective alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist perampanel (PER) in the treatment of LD. The drug appears to lead to sustained remission in myoclonus and GTCS.

Perampanel is highly selective, non-competitive alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid postsynaptic glutamate receptor antagonist. Activation of AMPA receptors by glutamate is thought to be responsible for excitatory synaptic transmission in the brain. Thus PER reduces neuronal hyperexcitation associated with seizures by targeting glutamate activity.

The efficacy and tolerability of PER has been demonstrated in well designed studies and it was approved as adjunctive therapy for drug-resistant partial seizures with or without secondary generalisation in patients with epilepsy<sup>7,8</sup>. Recommended dosage is 4-8 mg/day up to 12 mg/day<sup>9,10</sup>.

Here we report an open label, prospective study of 4 patients with genetically proved LD, treated with PER as add-on therapy.

## Methods

We studied 4 patients (2 males and 2 females), aged between 22 and 34 years (mean age 27.375 years). The diagnosis was confirmed by genetic analysis, all with mutation in NHLRC1 (EPM2B) gene.

These patients were previously included and reported in a clinical and genetic study of 14 LD patients from 10 families of Serbian/Montenegrin origin with more detailed clinical data presented in this paper<sup>11</sup>. The onset of the disease was between 11.5 and 14 years (mean age 12.75 years). The mean duration of the disease was 14.5 years (in the range of 8 and 21 years).

Patients with genetically confirmed LD were enrolled in our open label study after informed consent was obtained from patients and/or parents. The first patient was entered into the study in January 2015. Patients were assessed by both their treating physicians and parents prior to introduction of PER in order to obtain a comparative data.

**Table 2**

### Disability scale based on the residual motor and mental functions, daily living and social abilities<sup>13</sup>

1. Mild cognitive and motor impairment, preserved daily living activities and social interaction
2. Moderate mental decline, limitations in motor activities and limited social interaction
3. Severe mental and motor impairment, needing help in walking and regular assistance in daily living activity and poor social interaction
4. Patient wheelchair - bound or bedridden, and no significant daily living activities or social interaction

Therapy with PER started at the dose of 2 mg/day and was increased by 2 mg/day every 1–2 weeks. PER was titrated to an individual therapeutic dose depending on tolerability and clinical response, up to 12 mg/day. All concomitant AEDs, sodium valproate (4 patients), clonazepam (2 patients), levetiracetam (2 patients), phenobarbital and lorazepam (each in one patient) remained unchanged. Some adjustments of the AEDs dose regimen were made by the patient's treating physician when clinically indicated. After starting treatment with PER, its clinical efficacy was evaluated by comparing the seizure frequency and effect on myoclonus at the end of follow-up with those at the baseline. Parents were asked about the number of GTCS, experienced by the patients during the previous one-month period prior to evaluation time points. The averages and percentages of changes in GTCS frequency from the baseline period were calculated.

Follow-up period comprised 14–26 months with early termination in one patient due to the lack of efficacy.

Apart from recording the frequency of GTCS, parents were asked to assess: (a) myoclonus frequency, severity, amplitude, and intensity, and (b) the level of functional disability and cognitive performance.

We defined myoclonus as sudden jerks or twitches that occur in groups of muscles.

Myoclonus was assessed using numerical scales based on a modified version of the Unified Myoclonus Rating Scale (UMRS) (Table 1)<sup>12</sup>. Levels of ability across functional domains were assessed separately from myoclonus to determine the effects of PER on daily living tasks and to get a better picture of the disease stage for each patient.

**Table 1**  
Unified Myoclonus Rating Scale (UMRS)<sup>12</sup>

Intensity of myoclonus	
A.	Myoclonus frequency (0–5)
1.	no myoclonus
2.	only part of the day
3.	less than every 5 min
4.	once every 3–5min
5.	once every 1–2 min
6.	more than once a minute
B.	Myoclonus severity (0–4)
C.	Amplitude of myoclonus (0–3)
D.	Global assessment of intensity of myoclonus by patient caregiver (0–4)
Adjusted sum score: [(A + B + C + D) / 16 * 10]	

Adverse events (AEs) were reported throughout the study.

To assess the progression of the disease at PER introduction, we used Franceschetti's disability scale based on the residual motor and mental functions, daily living and social abilities (Table 2)<sup>13</sup>.

## Results

Molecular-genetic and clinical characteristics of patients are shown in Tables 3 and 4.

In all patients the previous antiepileptic therapy (sodium valproate, benzodiazepines, ethosuximide, levetiracetam, topiramate, zonisamide, primidone, piracetam and phenobarbital) was not effective. PER was gradually titrated and administered as add-on therapy at the doses of 6–8 mg once daily.

Four patients were enrolled with a mean age of 26.5 years. Two of the patients were females, and two were males. The mean dose maintained by patients at final evaluation was 8 mg/day. Two patients reduced their daily dose by 2 and 4 mg after reaching their maximum titrated dose of 10 mg daily, because of side effects (mood changes, agitation, increased hallucinations). By the end of the therapeutic response follow-up, three patients (pts. 1, 2, 3) had a greater-than-12-month exposure to PER treatment. One patient (pt. 4) discontinued treatment after 3 months of the treatment because of lack of efficacy for myoclonus. Patients were taken off the treatment at dosages of 6 mg (pt. 2), 8 mg (pts. 1 and 3), and 10 mg (pt. 4).

Compared to baseline, totally 3 patients of 4, showed improvement with introduction of PER. They had sustained reduction of myoclonus and almost complete disappearance in two patients (pts. 1 and 2) for shorter period of time (1-3

months). One patient (pt. 2), who was good responder initially, developed sleep disturbances, irritability and violent behavior on 8 mg/day. With dose reduction to 4 mg/day, side effect disappeared, but myoclonus was more pronounced. With dosage adjunction at 6 mg/day, the patient had no massive, erratic myoclonus, and only rarely was irritated. In one patient (pt. 3) PER was reduced after 2 months because of adverse effects, irritability and visual hallucinations. In one patient (pt. 4) PER was discontinued after 3 month of 10 mg/daily use, due to the lack of efficacy in myoclonus control.

Generalized tonic-clonic seizures were better controlled in all patients, two of them (pts. 1 and 2) had no GTCS for longer period of time, and other 2 had rare GTCS, with reduction for more than 50%. No aggravation of seizures was reported. The average number of GTCS per 28 days reported at baseline was 5 (range: 2–8). At the final evaluation the average number of GTCS was reduced to 1.0 (range 0–2).

Three patients (pts. 1, 2 and 3) had improvement in myoclonus. The average group adjusted score of myoclonus intensity at baseline was 6.56 compared with 2.97 and 2.5 at 3 months and 12 months, respectively (Table 5). There was no significant change in functional or cognitive measures. The mean adjusted score of functional disability at baseline was 3.5 and remained the same at the final scoring.

**Table 3**

### Molecular-genetic findings in patients with Lafora disease (LD)

Patient sex	Genetic mutation
1. Male	EPM2B (heterozygous, c.1048-1049delGA, deletion of the EPM2B gene)
2. Female	EPM2B (heterozygous, c.1048-1049delGA, deletion of the EPM2B gene)
3. Female	EPM2B (homozygous c.1048-1049delGA)
4. Male	EPM2B (homozygous c.1048-1049delGA)

**Table 4**

### Clinical characteristics of our patients with Lafora disease treated with adjunctive perampanel (PER)

Patient	Disease onset (years)	Disease duration (years)	Cognitive functioning	Disability level	Previous AEDs	Co-medication with PER	Age at PER introduction (years)	PER dosage (mg/day)	PER efficacy
1	13	21	severe decline	4	VPA, PRM, ZNS ketogenic diet, TPM LEV, CLZ, PB	VPA, LZP, PB	30	8	GTCS free, myoclonus reduced
2	12.5	17.5	severe decline	4	VPA, TPM ketogenic diet, LEV PB, CLZ	VPA, LEV	26.5		GTCS free, nearly stopped myoclonus
3	11.5	12	moderate decline	2/3	VPA, LEV, CLZ	VPA, CLZ	20	8	GTCS decreased, myoclonus reduced
4	12.5	8	IQ 65	3	CLB, TPM VPA, LEV, CLZ CLB, TPM	VPA, LEV, CLZ	20.5	10	non-responder

**IQ – intelligence quotient; AED – antiepileptic drug; VPA – valproic acid; LEV – levetiracetam; CLZ – clonazepam; CLB – clobazam; TPM – topiramate; PRM – primidone; ZNS – zonisamide; PB – phenobarbital; LZP – lorazepam.**

**Table 5**  
**Perampanel efficacy on seizures and myoclonus in Lafora disease (LD) patients**

Patient	No. of seizures/ 28 days <sup>b</sup> (Adjusted myoclonus score)	No. of seizures/ 28 days <sup>3</sup> (Adjusted myoclonus score)	No. of seizures/ 28 days <sup>12</sup> (Adjusted myoclonus score)
1	8 (3.75)	0 (0.625)	2 (1.25)
2	6 (7.5)	0 (0)	0 (2.5)
3	2 (6.25)	1 (3.75)	1 (3.75)
4	4 (8.75)	2 (7.5)	–
Average	5 (6.56)	0.75 (2.97)	1 (2.5)

<sup>b</sup> – baseline; <sup>3</sup> – 3 months after the drug introduction; <sup>12</sup> – 12 months after the drug introduction.

There were no differences between baseline and final functional abilities scoring (3.5). All of our patients had severe cognitive deterioration, with the average disease duration of 26 years. Two patients (pt 1 and 2) were bed-ridden (score 4 on disability scale, after average 9.5 years from the first symptoms) and had gastrostomy (after average 13.5 years from the first symptoms). Remaining two patients could walk only with assistance and had very reduced social life (score 3, after average 9.5 years from the first symptoms).

Adverse effects associated with PER treatment were reported in 3 of 4 patients. They included: sleep trouble, irritability, aggression, somnolence, impairment of vision, increased hallucinations, and headaches. No serious adverse effects were reported. They were rated mild to moderate and decreased or disappeared after the dose adjustment.

## Discussion

There is no effective therapy for LD. The inexorable progression and protracted suffering are agonizing to both patients and families. As Goldsmith and Minassian<sup>6</sup> stated, any extent of symptom relief is therefore highly desirable. Our study aimed to test the benefits reported in three separate case studies<sup>4-6</sup>.

Our patients had EPM2B mutation. As previously described, patients of Serbian/Montenegrin origin mainly have EPM2B mutation. This study suggests that mutations in the NHLRC1 gene may be a common cause of LD in the Serbian/Montenegrin population, primarily because of a founder effect<sup>9</sup>. We were encouraged by the publication of two case studies showing efficacy of PER<sup>4,5</sup> to use this medication in some of our patients. Our LD patients had limitations due to the high price and non-availability of the drug in Serbia. So, only small group of patients were able to use adjunctive PER.

In the meantime, new case series with 10 LD patients treated with PER was published<sup>6</sup>.

Our results are in general accord with the both single case and case series reports. A sustained and reproducible remission of myoclonus and GTCS was achieved with 8 and 10 mg of PER for a follow-up of six months in a 21-year-old woman with LD due to the homozygous missense mutation in exon 3 of the EPM2A gene (c.538CNG; p.L180V)<sup>4</sup>. In our 3 of 4 patients, both, seizures and myoclonus, improved

after the drug introduction. No favourable therapeutic effect of PER was seen in one case. Differently from previously published case studies except in one case, the response was impressive with near complete seizure reduction. Prevalence of the EPM2A patients were reported by Goldsmith and Minassian<sup>6</sup>. In another French-Serbian group of 8 LD patients with both, EPM2A (3 pts) and EPM2B (5pts) mutations, despite poorer cognitive and functional condition in EPM2B subgroup, no clear difference in the therapeutic response to adjunctive PER was noted<sup>14</sup>.

The (sub)continuous positive and negative myoclonus is especially disabling symptom in LD. According to evaluations and caregiver interviews, it appears that myoclonus did improve substantially in 3 of 4 of our patients.

Our case study was open-label and thus susceptible to biases.

Psychiatric and behavioural disturbances could be seen as adverse effects of PER. Patients with a history of psychiatric disorders may be at greater risk of developing anger, aggression, hostility, threatening behavior, homicidal ideation and irritability. LD patients with cognitive problems could be considered to be at greater risk for both, psychiatric and behavioral side effects.

Clinical recommendations were directed toward medications with broad spectrum efficacy in epilepsy, such as valproic acid, zonisamide and levetiracetam, and most clinicians refrain from using medications with activity restricted to focal seizures. PER was originally developed for focal-onset epilepsy, but recent studies have shown its spectrum strongly extended to generalized epilepsy<sup>15</sup> and our study appears to support this extension to progressive myoclonus epilepsy (PME), at least to LD.

The previous case studies reported improvements in functional abilities in LD patients treated by PER. In a case reported by Schorlemmer et al<sup>4</sup>, given daily dose of 10 mg, seizures stopped and the patient also regained her ability to walk with help and the aid of a walker. Dirani et al.<sup>5</sup> found striking improvement not only in myoclonus and seizure control but also in neurological functioning. Case series by Goldsmith and Minassian<sup>6</sup> showed no functional improvement. Observed adverse effects by caregivers were relatively mild and tolerable. No serious adverse effects were reported. However, side effects were severe enough for three patients

to withdraw from the treatment<sup>4</sup>. In our study, the functional and cognitive impairment maintained with no improvement, although the drug was introduced in later stages of the disease (3 and 4), after 9.5 years from the first symptoms.

A randomized study evaluating behavior, efficacy and safety of PER in adolescents with intractable focal seizures showed that most frequently reported adverse effects were dizziness [26 patients (30.6%) vs. placebo (14.6%)], somnolence [13 patients (15.3%) vs. placebo (4.2%)], and headache [nine patients (10.6%) vs. placebo (14.6%)]. Aggression was reported in seven patients receiving PER (8.2%) vs. 2.1% receiving placebo<sup>16,17</sup>.

Ultra-structural studies showed that the cytoplasm of dendrites at synapses are occupied or replaced by Lafora bodies (insoluble, malformed glycogen) suggesting a possible impact on synaptic function<sup>18</sup>. Perampanel was introduced as antiepileptic drug for partial-onset seizures. Its specific modulation of AMPA receptor, with noncompetitive binding at glutamate subreceptor, relatively independent of presynaptic transmitter release, is reducing neuronal excitability. It was shown that in LD, gamma-aminobutyric acid (GABA)ergic cortical neurons are reduced, due to neuronal loss and a specific damage in neurodevelopment of GABAergic neurons in the cerebral cortex as well<sup>19</sup>. Neurophysiology studies showed that hyperexcitability in LD is connected with severe impairment of inhibitory mechanisms<sup>20</sup>, though

impairment of astrocytic glutamate clearance was also suggested. Disrupted glycogen metabolism could explain important role of glutamate in LD hyperexcitability, since normal glycogen synthesis and breakdown are critical to the homeostasis of glutamate<sup>21, 22</sup>. Perampanel would likely confer benefit by diminishing neuronal network hyperexcitability, through its known AMPA antagonism and the balance of inhibitory to excitatory neurotransmitters<sup>5</sup>, not only for GTCS but also for cortical reflex myoclonus, commonly present in LD<sup>23</sup>.

Today we are step away from the curative therapy. Researchers are screening for small molecule inhibitors of glycogen synthase, they are using antisense oligonucleotides and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technics, developing gene and protein therapy<sup>24, 25</sup>.

### Conclusion

Perampanel introduced as add-on therapy in LD patients with advanced form of the disease, showed sustained remission in myoclonus and GTCS. Psychiatric side effects were dose related. In the close future the curative therapy will be available, but until then our small case series study supports previously published very rare observations that perampanel is beneficial new tool in the treatment of this severe epilepsy.

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## Isolated avulsion fracture of the fibular head

### Izolovani avulzioni prelom glave lisnjače

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#### Abstract

**Introduction.** Rupture of lateral collateral ligament of the knee is most often joined with other ligament ruptures. Isolated rupture of this ligament is rare and there are few papers about treatment options and results. Here we reported a case of isolated lateral collateral ligament rupture and the treatment outcome. **Case report.** A patient, 22 years old male, injured his left knee while playing American football. While landing on the outstretched left leg, he felt a sudden pain in his knee. The patient could not continue the competition. Initial orthopedic examination revealed lateral opening and further diagnostic procedure (magnetic resonance imaging) revealed isolated grade III rupture of lateral collateral ligament with avulsion fracture of the fibular head, and distension of anterior and posterior cruciate ligaments. Patient was surgically treated with metal sutures passed through conjoined tendon and proximal fibula. Postoperatively patient worn above knee cast for 6 weeks and after that he was included in rehabilitation. Three and six years after this injury, the patient has still been professional football player with no symptoms and no clinical instability of the knee despite radiological and computed tomography verified pseudoarthrosis of the fractured fibular head fragment. **Conclusion.** Early diagnostic and absence of additional injuries of the knee leads to a faster and full functional recovery of patients with isolated avulsion fracture of the fibular head, while surgical treatment provides knee stability with no residual ligament instability during sports activities.

#### Key words:

knee injuries; ligaments; fibula; fractures, avulsion; orthopedic procedures; athletic injuries.

#### Apstrakt

**Uvod.** Prekid spoljašnjeg bočnog ligamenta kolena najčešće je udružen sa prekidima drugih ligamenata. Izolovan prekid ovog ligamenta retko se sreće u kliničkoj praksi i u literaturi se nalazi svega nekoliko objavljenih slučajeva. Ovde smo prikazali jedan takav slučaj iz naše prakse sa načinom i ishodom lečenja. **Prikaz bolesnika.** Bolesnik, star 22 godine, zadobio je povredu levog kolena u toku igranja američkog fudbala. Povreda je nastala pri doskoku na ispruženo koleno, kada je osetio bol. Nije nastavio sa utakmicom. Inicijalni pregled kod ortopeda otkrio je postojanje spoljašnje bočne nestabilnosti, dok je naknadno uređena magnetna rezonanca pokazala postojanje izolovanog prekida spoljašnjeg bočnog ligamenta 3. stepena, sa avulzionim prelomom vrha glave lisnjače i postojanjem istegnuća prednjeg i zadnjeg ukrštenog ligamenta, ali bez prekida kontinuiteta vlakana. Bolesnik je operisan, pri čemu je fiksacija ulomka postignuta žičanim koncem. Postoperativno je postavljena imobilizacija natkolnim gipsom u trajanju od 6 nedelja, posle čega je pacijent uključen u rehabilitacioni tretman. Tri i šest godina posle povrede i dalje se profesionalno bavio američkim fudbalom, bez ikakvih subjektivnih tegoba kao i bez kliničke nestabilnosti kolena, i pored toga što su kontrolni radiogrami i kompjuterizovana tomografija pokazali postojanje pseudoartroze ulomka glave lisnjače. **Zaključak.** Rano postavljanje dijagnoze i odusustvo drugih povreda kolena bitni su preduslovi za brz i potpun oporavak ovih bolesnika, dok operativno lečenje obezbeđuje stabilnost kolena i povratak sportskim aktivnostima.

#### Ključne reči:

koleno, povrede; ligamenti; fibula; prelomi, avulzioni; ortopedске procedure; povrede, atletske.

#### Introduction

The fibular collateral ligament (FCL) is the primary varus stabilizer of the knee<sup>1,2</sup> and its injuries are frequently associated with anterior cruciate ligament (ACL), posterior cruciate ligament (PCL) and posterolateral corner injuries. Isolated grade III

tears of the lateral collateral ligament (LCL) with avulsion fracture of the fibular head are rare and these fractures have been called the “arcuate” sign<sup>3</sup>. The “arcuate” sign is used to describe an avulsed bone fragment related to the insertion site of the arcuate complex, which consists of the fabellofibular, popliteofibular, and arcuate ligaments<sup>4</sup>.

Although avulsion fracture of the fibular head associated with ACL, PCL and posterolateral corner injuries, is well described and primary repair has been recommended<sup>4-10</sup>, limited data exist in the literature on surgery techniques and clinical results<sup>11-13</sup>.

The purpose of this paper was to present results of surgical treatment of the patient with isolated grade III LCL injury with avulsion fracture of the fibular head.

### Case report

A patient, 22 years old, injured his left knee while playing American football. While landing on the outstretched left leg he felt a sudden pain in his knee. He could not continue the competition. In the emergency room, orthopedic surgeon noted the lateral opening of the knee during varus stress test. Lachman test could not be performed due to pain. Neurocirculatory status of the leg was normal. Four days later magnetic resonance imaging (MRI) of his left knee was made re-

vealing a distension of ACL and PCL (Figure 1), with no rupture, and avulsion of the head of the fibula (Figure 2).

Seven days after the injury, the patient was admitted to the hospital, and after four days more he was operated on. In the operating room, Lachman test was performed under general anesthesia and showed firm end point. Anterior and posterior drawer's tests were negative, and varus stress tests at 0 and 30 degrees of knee flexion were positive. After placing a tourniquet, a slightly curved skin incision was made on the lateral side of the knee from fibular neck to the level of lateral femoral epicondyle, and the fibular nerve was identified. Common attachment of biceps tendon and LCL to the dislocated fibular head fragment was prepared, and metal suture was passed through. One drill hole was placed through proximal fibula, 1 cm distal to the fracture level and the wire was pulled through this hole. After anatomic reposition of the fibular head fragment, the cerclage was tied with the knee in 30 degrees of flexion and neutral rotation. The tourniquet was deflated and bleeding was controlled. The wound was closed in layers with interrupted sutures.

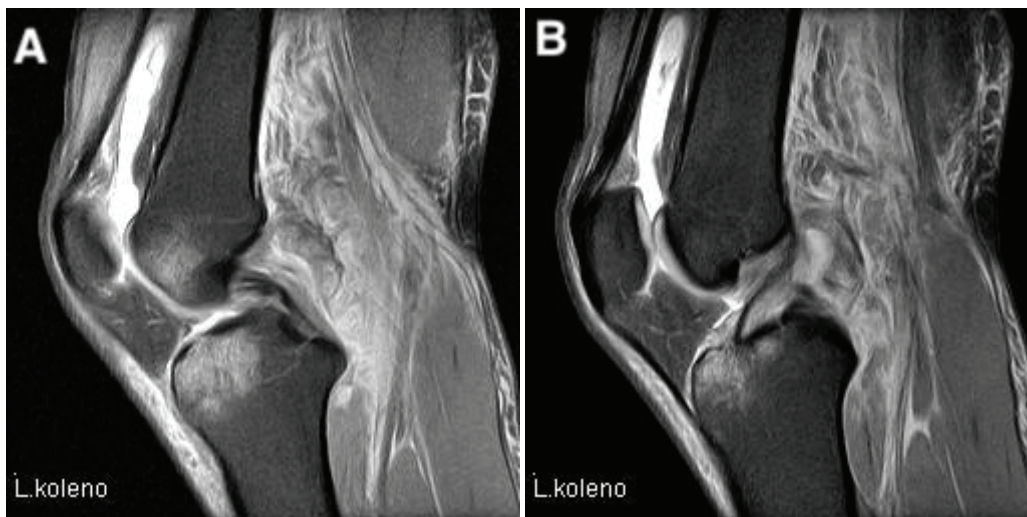


Fig. 1 – Preoperative magnetic resonance imaging (MRI): A) posterior cruciate ligament; B) anterior cruciate ligament.

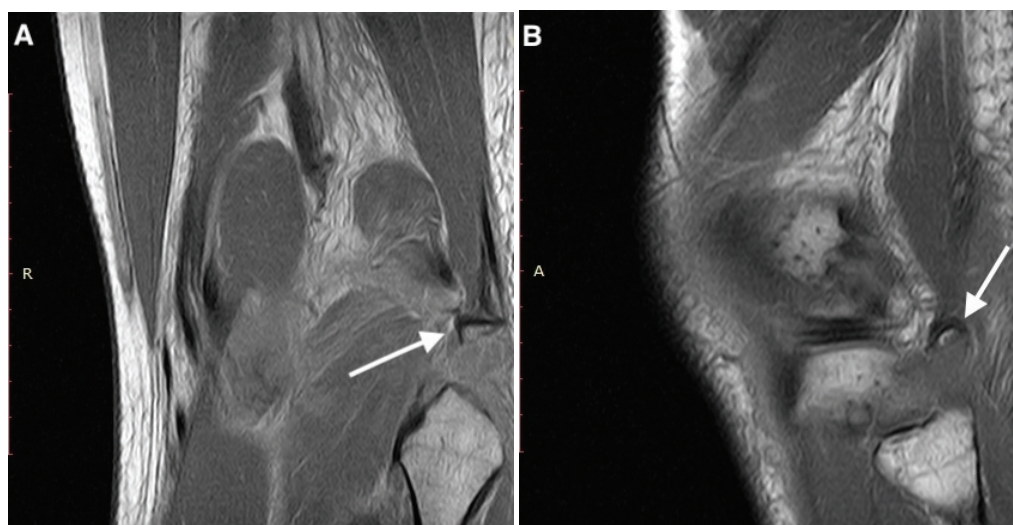
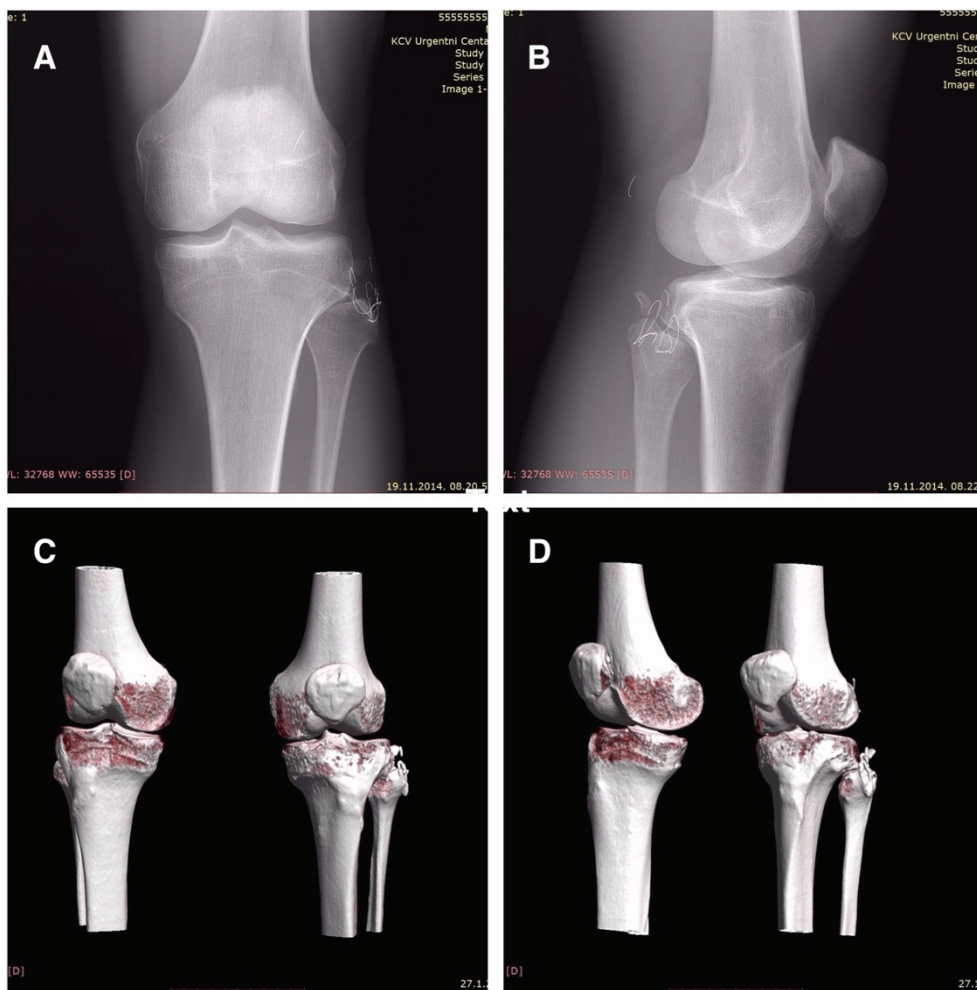


Fig. 2 – Preoperative magnetic resonance imaging (MRI): A) antero-posterior view of avulsed fragment; B) profile view of avulsed fragment.



**Fig. 3 – Postoperative radiography (X ray) and computed tomography (CT) findings:**  
**A) postoperative X ray finding, anteroposterior view; B) postoperative X ray finding, lateral view;**  
**C and D) postoperative CT scans.**

The above-the-knee plaster cast was applied. Six weeks later full weightbearing was allowed and physical therapy started. Six months after the injury, the patient was back to preinjury sports activity level.

On the follow-up 3 and 6 years later the patient was still professional American football player. He had full range of motion, with no pain and no swelling. Lachman test was with firm end point without differences in relation to the other knee. Anterior and posterior drawer's tests were negative, as on the other knee. Varus stress test was without lateral opening in despite of pseudoarthrosis of the fibular head revealed by radiography (X-ray) and computed tomography (CT) examination (Figure 3).

### Discussion

According to the literature, avulsion of the head of the fibula is described as a possible indicator of posterolateral instability of the knee mostly associated with other knee structure injuries<sup>14</sup>. These injuries can occur when anteromedial region of the tibia sustains direct hit while the knee is fully extended<sup>15</sup>. Rarely, grade III of LCL tears can be isolated and these result from forces of lower magnitude<sup>3</sup>.

High intensity force applied to the knee usually affects additional structures in the area. Only Phadnis et al.<sup>16</sup> reported isolated avulsion fracture of the fibular head which occurred during a primary total knee replacement.

Physical examination right after injury does not have any specific findings. Specific orthopedic examination most often reveals posterolateral instability of grade 2 or more. These specific clinical tests are: varus opening at 20 degrees, posterolateral drawer test, external rotation at 30 degrees and 90 degrees (Dial test), and the reverse pivot shift. Next step should be making anteroposterior (AP) and lateral X ray which could reveal so-called arcuate sign a small fragment of the proximal fibula. As to the results of Huang et al.<sup>15</sup> this fractures fragment most often is horizontally oriented, no more than 1 cm in length, and displaced medially and superiorly by traction of conjoined lateral collateral ligament and biceps femoris tendon. This small fragment usually is overseen and masked by other knee joint bony structures, but CT scan or MRI can easily visualize its size, and adjacent soft-tissue injuries as we did in this case.

When isolated grade III LCL rupture is present, further treatment could be operative or non-operative. Bushnell et

al.<sup>3</sup> compared operatively and nonoperatively treated nine football players with grade III isolated LCL injuries. As to results, both groups had good functional outcome, but those players treated nonoperatively returned to play 9 weeks earlier. In the operated group three out of four patients had avulsion fracture of the fibular head, and in non-operative group only one out of five. Phadnis et al.<sup>16</sup> reported isolated avulsion fracture of the fibular head treated nonoperatively with good results. This fracture occurred during a primary total knee arthroplasty with no late varus instability.

Avulsion fractures of the proximal fibula are well described and primary repair has been recommended when occurring with other knee injuries. However, very limited data exist in the literature about surgical technique and clinical results of isolated LCL injuries. Our patient was professional football player with clinically significant knee instability on

varus stress test and that is why we decided to treat him operatively. We didn't want to take chance of having remaining knee instability in young professional football player. Fixation was achieved with metal sutures and in despite of pseudoarthrosis of avulsed fragment, there was no clinically instability of knee, and he continued playing football for many years after with no complaint.

### Conclusion

Early diagnostic of isolated fibular head fracture enables adequate treatment, and anatomic reduction of avulsed fragment is achieved easily. Absence of additional injuries of the knee leads to faster and full functional recovery of these patients with no residual ligament instability.

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## Primary percutaneous intervention of the right coronary artery in a setting of anomalous origination of left coronary artery from the opposite sinus of Valsalva

Primarna perkutana intervencija desne koronarne arterije u okolnostima anomalnog porekla leve koronarne arterije iz suprotnog sinusa Valsalva

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### Abstract

**Introduction.** Coronary artery anomalies are infrequent but anticipated findings during percutaneous coronary interventions (PCI). Compared to consistent reporting in angiographic series, they seem to be underreported in interventional studies, and particularly in the setting of primary PCI, where their prompt recognition is of the utmost importance. **Case report.** We present a 50 years old male with inferior ST-elevation of myocardial infarction (STEMI) and right ventricular involvement with solitary ostium for all three coronary arteries in the right aortic sinus of Valsalva. The patient had an extremely rare variant of coronary artery origination belonging to the type A4b2c2 of Angelini's classification. Correspondingly, it belongs to the left Anomalous origination of a Coronary Artery from the Opposite Sinus of Valsalva (ACAOS) class with the intraseptal course of left anterior descending artery. We managed successfully to implant a drug eluting stent in the proximal right coronary artery in a lengthy and stormy procedure, with the need for guiding catheter exchange, temporary pacing and dealing with no-reflow phenomenon. **Conclusion.** We summarize diagnostic hints for angiographic recognition of dominant variants of the left ACAOS and practical aspects of performing PCI in such patients. Also, we debate on the functional significance of coronary anomalies and its further implications from the prognostic and therapeutic aspects. We propose adoption of the novel classification of coronary anomalies of Angelini's group in the routine clinical practice. Finally, we call for the inclusion of specific training in coronary artery anomalies into the interventional cardiology fellowship curriculum.

### Key words:

percutaneous coronary intervention; coronary vessel anomalies; no-reflow phenomenon; st-elevation myocardial infarction.

### Apstrakt

**Uvod.** Anomalije koronarnih arterija nisu čest, ali su očekivani nalaz tokom perkutanih koronarnih intervencija (PCI). Za razliku od relativno redovnog prijavljivanja koronarnih anomalija pri dijagnostičkim koronarografijama, one se nedovoljno često prikazuju u izveštajima sa interventnih procedura, posebno u sklopu primarne PCI, kada je upravo njihovo promptno otkrivanje od najveće važnosti. **Prikaz bolesnika.** Prikazan je muškarac, star 50 godina, sa akutnim infarktom miokarda sa ST elevacijom (STEMI) donjeg zida koji je zahvatio i desnu komoru, kod koga je nađen zajednički ostijum za sve tri koronarne arterije u desnom aortnom sinusu Valsalve. Ova anomalija je ekstremno retka varijanta ishoda koronarnih arterija koja odgovara tipu A4b2c2 Angelinijeve klasifikacije. Istovremeno, ona pripada i kategoriji abnormalnog ishoda koronarnih arterija iz kontralateralnog sinusa Valsalve (ACAOS) sa intraseptalnim kursom proksimalnog dela prednje descendente arterije. Kod bolesnika je uspešno ugrađen jedan stent obložen lekom, tokom produžene i turbulentne procedure, uz potrebu izmene katetera vodiča, upotrebu privremenog *pacemaker-a* i pojavu fenomena usporenog koronarnog protoka po otvaranju arterije. **Zaključak.** Detaljno su prikazani dijagnostički postupci za angiografsko prepoznavanje dominantnih varijanti abnormalnog ishoda koronarnih arterija iz kontralateralnog sinusa Valsalve, kao i praktični aspekti izvođenja perkutanih intervencija kod ovakvih bolesnika. Analizirani su funkcionalni značaj koronarnih anomalija i njihove implikacije na dalju prognozu i terapijski pristup. Predloženo je usvajanje nove klasifikacije koronarnih anomalija, predložene od Angelinijeve grupe, u rutinskoj kliničkoj praksi. Na kraju, potrebno je uključivanje specifične obuke o svim relevantnim aspektima koronarnih anomalija u redovni program obuke interventnih kardiologa.

### Ključne reči:

perkutana koronarna intervencija; koronarni krvni sud, anomalije; fenomen odsustva ponovnog protoka; infarkt miokarda sa st-elevacijom.

## Introduction

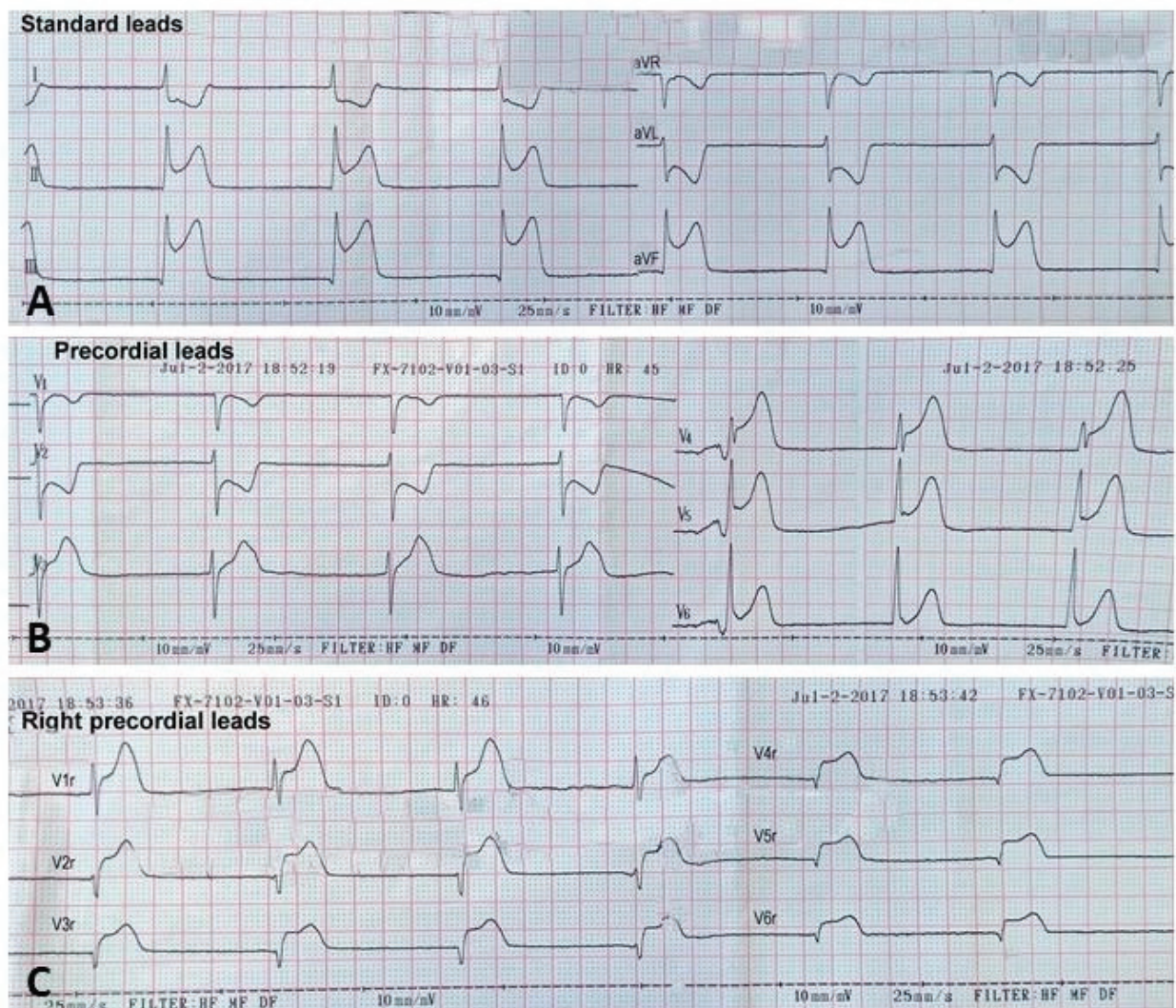
Anatomic variants of coronary artery origin and course occur infrequently, but with the consistent prevalence in different series of patients. Misdiagnosing of unsuspected aberrant origin of coronary arteries is a potential problem for busy operator leading to erroneous conclusion that the artery is occluded, been responsible for the prolongation or failure of the procedure. Particularly, it is important to have this entity in mind when dealing with acute coronary syndromes since the culprit lesion may be missed.

## Case report

### Clinical presentation

Fifty years old male was self-referred to the local emergency department because of the acute severe chest pain of prolonged duration followed by a brief syncopal episode. His risk factors were: hypertension, hypercholesterolemia, heavy smoking and positive family history (his father died of heart

attack at the age of 50). On admission, 45 minutes from pain onset, electrocardiogram (ECG) showed inferolateral ST elevation myocardial infarction (STEMI) with intermittent nodal rhythm (Figure 1, A and B), and right precordial leads revealed the right ventricular involvement (Figure 1, C). Patient received loading antiplatelet therapy (ASA 500 mg and ticagrelor 180 mg) and was immediately transferred by the cardiology ambulance to our facilities for primary percutaneous coronary intervention (PCI) within 90 minutes of the first medical contact. During the transfer, the patient was bradycardic and hypotensive despite 1.5 liter of volume expansion; chest pain and ST elevation were persistent. On admission his blood pressure (BP) was 90/55 mm Hg, heart rate (HR) 48 bpm, ST elevation persisted in the same leads with transient nodal rhythm. Echocardiographic examination revealed inferolateral hypokinesia with the global left ventricular ejection fraction of 45%, and a trace of mitral regurgitation. On the catheterization table, immediately before arterial puncture, ECG showed ST elevation resolution followed by pain relief.



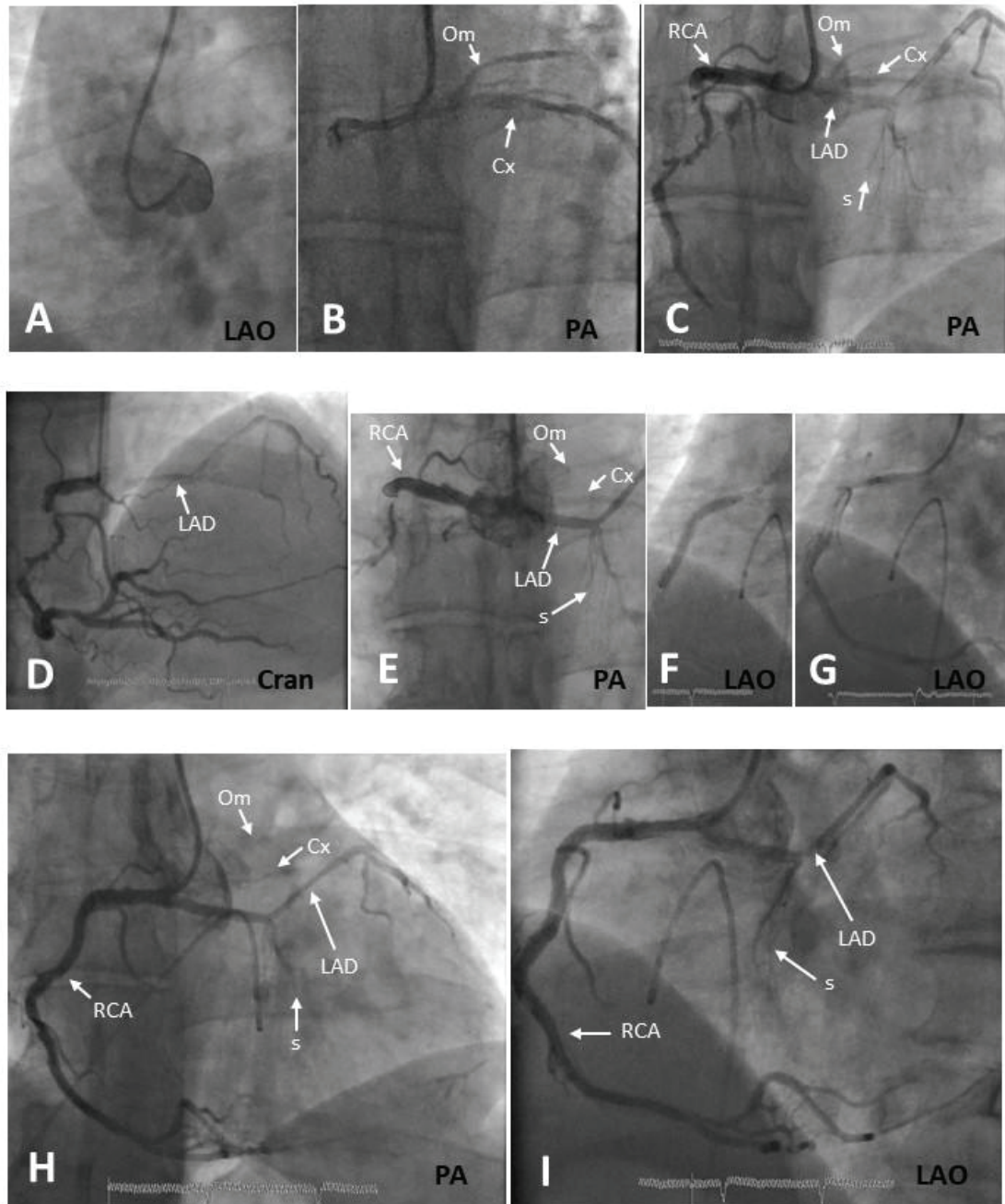
**Fig. 1 – Electrocardiography (ECG) at presentation: acute inferolateral myocardial infarction (A and B), with right ventricular infarction (C), nodal rhythm.**

### Interventional procedure

Coronary procedure was done by the radial approach. Patient was heparinized with 100 IU/kg of unfractionated heparin (UFH). Because of bradycardia and intermittent nodal rhythm, a temporary pacemaker electrode was inserted by the femoral vein and left in a standby position. Accelerated volume substitution was continued by the i.v. line. Judkins

Left 5F diagnostic catheter failed to cannulate left coronary artery in the left aortic sinus of Valsalva; non-selective contrast injection confirmed the absence of coronary ostium and the originating artery (Figure 2A).

Hockey Stick 6F SH (side holes) guiding catheter visualized solitary coronary ostium in the right sinus of Valsalva with the anomalous origin of all three main coronary vessels (Figure 2B and C).



**Fig. 2 – Angiographic images of the procedure: A) absence of coronary ostium in left sinus of Valsalva; B) sub-selective cannulation showing anomalous circumflex with caudal-posterior loop; C) non-selective cannulation showing all three coronary arteries originating from right sinus of Valsalva. Note the typical “eye” configuration between left anterior descending (LAD) (inferior) and circumflex (Cx) artery (superior) and proximal septal branches (S), LAD is showing caudal-anterior loop; same phenomenon can be appreciated at images E and H; D) subocclusive stenosis of right coronary artery (RCA) with substantial thrombotic burden; E) wire negotiating at subocclusive stenosis; F) coronary stenting; G) no-reflow phenomenon; H) and I) final result. Note the “hammock” appearance of the proximal LAD (also seen at images B, C, D and E) and proximal septal branches indicating septal trajectory.**



Guiding catheter was then sub-selectively positioned into the dominant right coronary artery (RCA) revealing subocclusive, eccentric and heavily calcified stenosis at the proximal, bending part of RCA (Figure 2D). Distal flow in RCA was thrombolysis in myocardial infarction (TIMI) 2 grade, with spotty intraluminal filling defect distally to stenosis suggestive of acute thrombotic burden. Crossing of stenosis was initially attempted by Whisper<sup>®</sup> coronary guidewire, failing to negotiate the angulated course at the entry of stenosis (Figure 2, E). The same applied for the next attempt with Runthrough<sup>®</sup> NS coronary guidewire (the former wire was anchored in the conal branch for better support). Whole system was then replaced by AmplatzAL 2, 6F SH guiding catheter providing more selective cannulation (Figure 2F to I) and better backup enabling easy passage of ASAHI Sion Blue<sup>®</sup> coronary guidewire and its positioning in the distal RCA branch. Passage of the wire completely reoccluded the artery followed by the abrupt ST re-elevation and hemodynamic collapse (BP 50/30 mmHg with the development of complete AV block initiating ventricular pacing) necessitating rapid actions. Prompt lesion predilatation was done with noncompliant 2.5/20 mm balloon at 16 atmospheres followed by implantation of 3.5/25 mm Orsiro<sup>®</sup> stent at 14 atmospheres (Figure 2F). Immediate coronary angiography revealed no-reflow phenomenon in the targeted RCA (Figure 2G). Hemodynamic status improved marginally but remained unstable. Intracoronary bolus with 120 mcg of adenosine was injected distally to the implanted stent through the Finecross<sup>®</sup> MG microcatheter. That was followed by the normalization of coronary flow and gradual hemodynamic recovery (Figure 2H and I). At that point, an intracoronary high-dose bolus of tirofiban (Ag-grastat<sup>®</sup>) was administered over 3 minutes (25 mcg/kg), followed by the continuous tirofiban infusion (0.15 mcg/kg/min) for the following 12 hours because of the substantial preexisting thrombotic burden.

Overall, the door-to-balloon time was less than 40 minutes; whole procedure was done utilizing 140 mL of non-ionic contrast media, with 21 minutes of fluoroscopy time and total radiation exposure dose of 1,950 milligray (mGy). Patient remained stable following transfer to the coronary care unit where temporary pacemaker lead was removed.

During the remaining hospital stay, plateaued enzyme release and accelerated washout was observed, followed by the improvement of baseline wall motion abnormalities on control echocardiographic examination. Patient was discharged on standard post myocardial infarction therapy with particular attention to the continuation of beta blockers. He had an uneventful clinical course over the 6-month follow-up, avoiding extreme exertions, but refusing additional stress testing or further invasive reevaluation.

## Discussion

### *Prevalence and classification of coronary artery abnormalities*

The prevalence of coronary artery anomalies ranges from 0.5% in a multicenter pediatric autopsy study from 1994<sup>1</sup> to 1.3% in the study reported from the Cleveland

Clinic in 1990 among 126,595 patients undergoing coronary arteriography in which anomalies of origin and distribution occurred in 87% and coronary artery fistulae in 13%<sup>2</sup>. The similar prevalence of 1.34% was reported in a coronary arteriography study on a Central European population<sup>3</sup>. In a series of 12,457 adult coronary angiographies from Turkish population Yildiz et al.<sup>4</sup> reported 0.9% of prevalence of coronary artery anomalies, with 89.3% being anomalies of origin and distribution and 10.7% coronary fistulae.

Up to the present, several classifications of coronary artery anomalies have been proposed. The traditional classification of abnormal origin and distribution of coronary originates from Lipton et al.<sup>5</sup> in 1979. Being practical, but incomplete, this classification was further improved by Yamanaka and Hobbs<sup>2</sup> in 1990. In 1993, Shirani and Roberts<sup>6</sup> proposed atomically based classification of congenital coronary artery abnormalities, depending on the originating aortic sinus and the pattern of distribution of coronary arteries<sup>7</sup>. The latest classification came from Angelini's group in 2007<sup>8</sup>. It is based on their long-lasting experience starting in 1999 by the analysis of 1,950 consecutive angiograms with adoption of strict criteria for assessing coronary normality versus abnormality<sup>9</sup>. This approach was refined and elaborated in subsequent publications dealing with pathophysiology and clinical relevance of coronary anomalies<sup>10, 11</sup>, as well as with novel diagnostic aspects in adults<sup>12-14</sup>. Coronary artery abnormalities are classified into 4 categories: A) anomalies of origination and course; B) anomalies of intrinsic coronary arterial anatomy; C) anomalies of coronary termination; and D) anomalous anastomotic vessels. This classification starts with definition of normal coronary anatomy, making distinction between relevant arteries and smaller ones (such a conal branch), and defining that any form observed in > 1% of unselected general population is considered as normal. Also, it incorporates the concept of anomalous origination of a coronary artery from the opposite sinus of Valsalva anomalous coronary artery from the opposite sinus (ACAOS), being lately in the focus because of its clinical and prognostic consequences.

Ectopic left coronary artery (or LAD) arising from the right coronary sinus may have 4 possible paths: 1) anterior to pulmonary outflow (free-wall), 2) interarterial, (between aorta and pulmonary artery), 3) intraseptal (intramural, or sub-pulmonary), 4) retro-aortic (or posterior). Ectopic circumflex arising from the right aortic sinus may have the following courses: 1) posterior atrioventricular groove or 2) retro-aortic.

Overall, our patient had a rare variant of abnormal origin of coronary arteries corresponding to Angelini classification type A4b2c2. Also, it fits into Shirani and Roberts II D3 class, Yamanaka R-II S class or LiptonR III class. Anatomically it belongs to the category of left ACAOS with intraseptal course of left anterior descending artery.

### *Current diagnostics of coronary artery anomalies*

Selective coronary arteriography remains the gold standard for diagnosis of coronary artery anomalies, but occa-

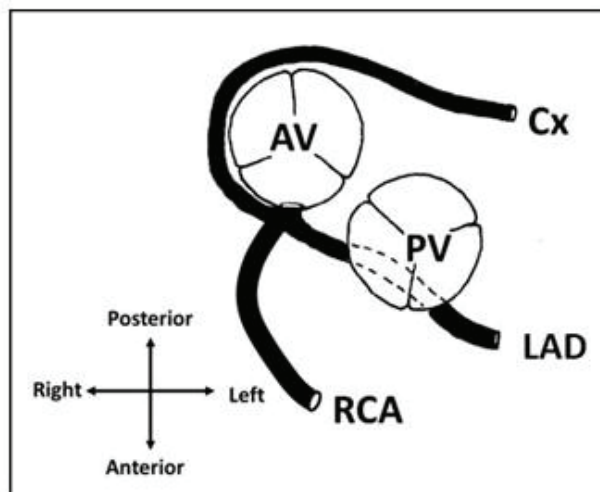
sionally it may fail to identify the proximal course of coronary arteries, where multislice computed tomography (MSCT) gives more detailed information on the proximal tract of coronary arteries and their relationship with the surrounding structures<sup>15</sup>. It was shown recently that the prevalence of coronary anomalies is substantially higher when diagnosed by MSCT than with coronary angiography, even after exclusion of myocardial bridging<sup>16</sup>.

However, radiation exposure and invasiveness are favoring nowadays the application of cardiovascular magnetic resonance (CMR) imaging for diagnosing coronary artery anomalies, particularly in children and young adults. Coronary artery anomalies were reported in 0.3% of 59,844 CMR scans performed over 15-year period in a multicenter study with valuable prognostic information over 4.3 years median follow-up<sup>17</sup>.

Since most of coronary artery anomalies are diagnosed incidentally during coronary angiography, it is important to recognize them and to make correct angiographic identification, however that requires specific training of the operator<sup>12</sup>. Unfortunately, it may happen – mainly during primary angioplasty when operators are focused on opening an occluded artery and respecting the need for rational usage of contrast media – that acquired angiographic data do not allow correct identification and the precise reconstruction of the proximal course of anomalous vessels. However, a long time ago, precise instructions how to obtain angiographic identification of coronary artery anomalies have already been proposed. In 1985, Ishikawa and Brandt<sup>18</sup> presented a practical “cookbook” for diagnosing all four dominant types of anomalous origin of the left coronary artery from the right aortic sinus of Valsalva based on two orthogonal angiographic projections: right anterior oblique (RAO) and left anterior oblique (LAO), focusing on the orientation of the proximal loop in the frontal and sagittal planes. This concept was further simplified by Serota et al.<sup>19</sup> in 1990 providing the same identification of anomalous origin and proximal course of anomalous coronary arteries from a single RAO projection. Serota’s method provides helpful diagnostic hints for facilitating this process using evocative “dot and eye” signs to distinguish easily between four main types of anomalous course. In addition, Chaitman et al.<sup>20</sup> proposed an useful diagnostic tip for distinguishing intraseptal course of left coronary artery originating from the right sinus of Valsalva (considered as benign variant) from the interarterial course: the presence of septal perforators in the proximal part of the vessel, preferably in the lateral and left anterior oblique projections. More demanding angiographic model, utilizing additional contrast, has been proposed by Wang et al.<sup>21</sup> in 1997. It is using simultaneous biplane coronary and pulmonary arteriography (RAO 30 and LAO 60) to define the course of anomalous left coronary artery originating from the right sinus of Valsalva. Levophase imaging of the ascending aorta additionally increases its diagnostic utility. Digital subtraction can further improve this procedure by enhancing levophase images of coronary artery-ascending aorta relationship<sup>21</sup>. An useful diagnostic hint by placing a catheter into the pulmonary artery to help in determining the

course of anomalous coronary artery in relation to the pulmonary artery was proposed by Chu and Cheitlin<sup>22</sup> in 1993 allowing confident delineation of the interarterial variant from the other, benign forms. Also, if using pacing lead during the percutaneous procedure, like in our case, it may be positioned into the right ventricular outflow tract and utilized as a reference marker to aid in determining the proximal course of anomalous coronaries.

In our patient, shortly after failing to cannulate left coronary artery, we confirmed the absence of its ostium in left aortic sinus of Valsalva by sub-selective had injection of radiographic contrast (Figure 2A). When discovered ACAOS, we used orthogonal projections to determine the proximal course of anomalous arteries, following recommendations of Ishikawa and Brandt<sup>18</sup>, and diagnostic hints by Serota et al.<sup>19</sup>. In addition, we confirmed our finding of the intraseptal course of left anterior descending (LAD) artery by the presence of septal perforators according to the findings of Chaitman et al.<sup>20</sup>. It is important to note the “hammock” appearance of the proximal course of LAD<sup>2</sup> at the Figure 1 images B, C, D, E, H and I confirming the septal course with caudal-anterior loop. On the other hand, circumflex (Cx) artery is taking the course behind the aorta and anterior to the atria to the left, making caudal-posterior loop. Similarly, image C at the Figure 2 is showing typical “eye” configuration between LAD (inferior) and Cx (superior) confirming, together with prominent septal (s) branches, the septal course of proximal portion of LAD<sup>19,20</sup>. Schematic presentation of the origin and proximal course of main coronary arteries in our case is provided on the Figure 3.



**Fig. 3 – Schematic drawing: origin and proximal course of coronary arteries in the presented case. AV – aortic valve; PV – pulmonary valve; LAD – left anterior descending artery; RCA – right coronary artery; Cx – circumflex artery.**

#### *Clinical and functional significance*

Precise definition of the origin and course of anomalous coronary arteries is important when planning coronary revascularization, either by means of surgery or coronary angio-

plasty. In a case of primary angioplasty where the “time is muscle”, there is an even greater need for accurate and timely diagnostics.

Presence of certain types of anomalous coronary arteries has important prognostic implications. It has been shown that coronary artery anomalies may cause clinically disabling symptoms, especially in young adults, including dyspnea, angina pectoris and syncope<sup>14</sup>. Furthermore, such conditions are associated with increased frequency of myocardial ischemia and sudden cardiac death in young persons, especially during or immediately after strenuous exertion<sup>14</sup>. Maron et al.<sup>23</sup> followed a large registry, assembled over a 27-year period, of sudden deaths among US competitive athletes reporting constantly that after hypertrophic cardiomyopathy, anomalous origin of coronary artery from the opposite sinus of Valsalva is the second most common cause of sudden death in young competitive athletes in the USA<sup>24</sup>. Of 1,866 athletes who died suddenly (or survived cardiac arrest) over that period, hypertrophic cardiomyopathy was a cause in 36% of cases and congenital coronary artery anomalies in 17%<sup>23</sup>. Eckart et al.<sup>25</sup> was following military recruits in USA aged 18 to 35 years, finding 126 nontraumatic deaths, 64 of them were attributed to cardiac causes. An anomalous coronary artery accounted for one third of cardiac cases (21 of 64 cases), and in each of them left coronary artery originating from the right sinus of Valsalva was taking interarterial course between the pulmonary artery and aorta<sup>25</sup>. In that study, ACAOS was a more-common cause of cardiac death (61%) than cardiomyopathies (36%). Similarly, studying the sudden unexpected death in persons less than 40 years of age in Israel, where autopsy is obligatory in such cases, Drory et al.<sup>26</sup> in a series of 162 consecutive cases found that incidence of sudden death related to anomalies of coronary arteries was 0.6%.

Although anomalous origin of the right coronary artery is four times as common as anomalous origin of left, sudden death occurs more frequently in patients with anomalous origin of left coronary artery<sup>27</sup>. Interarterial course of anomalous artery originating from the contralateral sinus between pulmonary and aortic roots is held responsible in most cases, although exact mechanism is unclear. Traditionally, “scissor-like” mechanism was proposed, where the expansion of great vessels during vigorous exercising is compressing anomalous artery along its interarterial course and provoking ischemia. Also, it was proposed that expansion of aorta and pulmonary artery during exercise may provoke spasm, or kinking and torsion, of anomalous artery along its aberrant interarterial course. Presence of coronary ostial ridge which may function as valve, restricting flow during exertion has been proposed, as well. Less supported by evidences is the suggestion that anomalous origin may be associated with accelerated atherosclerotic disease, or that the congenitally small left coronary artery is responsible for ischemia. Novel insights in coronary anomalies with origination from the contralateral sinus of Valsalva (ACAOS) by intravascular imaging<sup>10-14, 28, 29</sup> are discovering that the proximal portion of aberrant vessels runs within the aortic wall (intramural, or intraparietal course)<sup>30</sup> being often hypoplastic and exposed to lateral compression

forming a slit-like formation that compromises the flow. During vigorous activity this proximal part is susceptible for further collapsing or closing provoked by the increase of cardiac stroke volume and arterial hypertension.

#### *Reports of primary PCI in anomalous coronary anatomy*

There is a limited number of publications dealing with coronary interventions in patients with anomalous origin of coronary arteries, most of them being case reports. Data on primary PCI in this setting are even more scarce. However, most of authors report prolonged procedures, with higher than usual complication rates and occasionally with adverse acute outcome, particularly in the case of primary procedures.

In our patient, the cannulation or the coronary ostium was challenging, requiring interchange of guiding catheters for proper alignment and better support, as well as multiple wire manipulations to pass initial sharp bends. For that purpose, there are several reports of successful utilization of “mother and child” sub-catheter technique for selective cannulation of coronary ostium in the percutaneous treatment of coronary anomalies. Also, in order to avoid proximal dislodgment of thrombotic material and eventual massive embolization into adjacent arteries originated from the same ostium, we did not perform thrombo-aspiration.

Overall, in our case the primary PCI had a stormy course with favorable outcome, however with higher fluoroscopy time, contrast utilization and radiation exposure compared with our median values. During the index procedure, we managed angiographically to assess the course of proximal segments of all coronary arteries confirming the intraseptal trajectory of LAD consider as prognostically favorable anatomic variant. However, based on our findings, as well as on the history of sudden cardiac death of his father in his middle age, we have suggested more watchful clinical follow-up by means of repeated exercise stress testing and ECG Holter monitoring along with the avoidance of extreme physical activities.

#### **Conclusion**

Altered coronary anatomy mandates prompt recognition imposing higher procedural risk and requesting technically more demanding approach during percutaneous procedures. It is important to select appropriate guiding catheter providing sufficient support without pressure dumping and to prevent coronary dissection, being particularly threatening complication in this scenario. When performing PCI in the setting of anomalous coronary origin, operator may expect sharp bends or acute take-offs prolonging the procedure and requiring specific skills. It is important to perform meticulous measures to avoid thromboembolism or proximal thrombotic propagation into the adjacent coronary arteries originating from the same ostium. Likewise, such cases may need more aggressive antiplatelet therapy balanced by the individualized hemorrhagic risk. Operator should anticipate a higher than usual chance of hemodynamic instability, reperfusion disorders, as well as rhythm and conduction distur-

bances. For that reason, each coronary anomaly should be evaluated individually weighting its potential functional and prognostic significance and making decision about the need for specific therapy.

When facing with anomalous origin of coronary arteries, operators should do their best to define the precise anatomy and their exact trajectory. That requires specific aware-

ness to coronary anomalies, so that we call for the inclusion of specific training in coronary artery anomalies into the interventional cardiology fellowship curriculum. Based on current experience, from the diagnostic and prognostic standpoint, we propose adoption and adherence in reporting with the latest classification of coronary artery anomalies from Angelini's group.

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## Secretory breast carcinoma in adulthood – A case report with literature review

### Sekretorni karcinom dojke u odraslom dobu

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#### Abstract

**Introduction.** Secretory breast carcinoma is rare subtype of breast carcinoma which occurs primarily in children and young adults, so in the past it was called juvenile carcinoma.

**Case report.** A 67-year-old female patient presented with mass of the right breast since one month. After physical, routine laboratory examination and mammography, core needle biopsy was performed and histopathological examination confirmed invasive carcinoma. Immunohistochemically, estrogen-receptors (ER) and progesteron-receptors (PR) showed weak positive reaction in 10% of tumor cells, while human epidermal growth factor receptor-2 (HER-2) was without expression. After an adequate preoperative preparation, operation was done – quadrantectomy with sentinel lymph node biopsy. Postoperatively, the patient was treated with 6 cycles of cyclophosphamide, methotrexate and fluorouracil (CMF) combination, radiotherapy (60 Gy) and tamoxifen. After 5-year follow-up the patient had no signs of the disease. **Conclusion.** Secretory breast cancer is a rare subtype of invasive breast carcinoma with wide age range of occurrence and good prognosis despite its triple-negative immunophenotype. Although the therapeutic management is non-consensual for this breast cancer special type, surgery is considered the mainstay of the treatment as well as the adjuvant chemotherapy and radiation.

#### Key words:

breast neoplasms; secretory breast carcinoma; triple negative breast neoplasms; diagnosis; mastectomy, segmental; adult.

#### Apstrakt

**Uvod.** Sekretorni karcinom dojke je redak tip karcinoma dojke koji se prvenstveno javlja kod dece i mladih odraslih osoba, zbog čega se u prošlosti nazivao juvenilnim karcinomom. **Prikaz bolesnika.** Žena, starosti 67 godina, javila se zbog palpabilne, bezbolne mase u desnoj dojci, koja se javila mesec dana pre prijema. Nakon fizikalnog pregleda, rutinskih laboratorijskih analiza i mamografije, načinjena je iglena biopsija i patohistološkom analizom dijagnostikovana je invazivni karcinom dojke. Imunohistohemijski, 10% tumorskih ćelija je pokazalo slabu pozitivnost na estrogenske i progesteronske receptore, dok je receptor humanog epidermalnog faktora rasta-2 (HER-2) bio negativan. Posle adekvatne preoperativne pripreme, izvršen je operativni zahvat – kvadrantektomija sa biopsijom limfnog čvora stražara. Postoperativno, bolesnik je tretiran kombinacijom ciklofosfamid/metrotreksat/fluorouracil (6 ciklusa), radioterapijom (60 Gy) i tamoksifenom. Petogodišnjim praćenjem bolesnice nisu ustanovljeni znaci bolesti. **Zaključak.** Sekretorni karcinom dojke je redak tip karcinoma dojke, koji zahvata sve starosne grupe i ima dobru prognozu bez obzira na karakteristični tripl-negativni fenotip. Iako ne postoji standardizovan protokol lečenja, hirurška resekcija uz adjuvantnu hemoterapiju i zračnu terapiju su glavni terapijski postupci za ovaj tip karcinoma dojke.

#### Ključne reči:

dojka, neoplazme; dojka, sekretorni karcinom; dojka, neoplazme, tripl negativne; dijagnoza; mastektomija, segmentalna; odrasle osobe.

#### Introduction

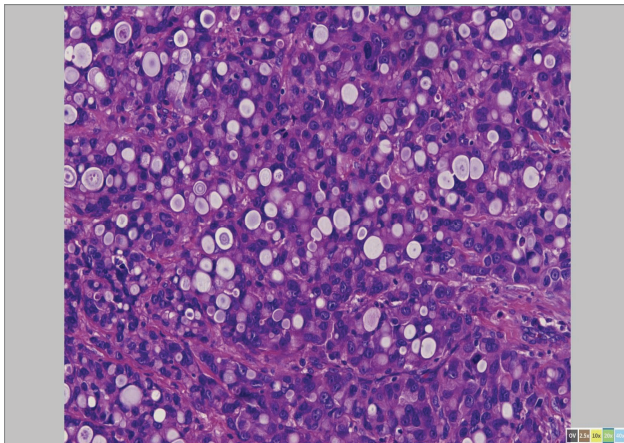
Secretory breast carcinoma (SBC) is rare subtype of breast carcinoma (< 0.1%). This subtype of breast carcinoma

occurs primarily in children and young adults, though persons of any age may be affected. Grossly, it is well-circumscribed tumor, which is microscopically composed of glands and solid nests with microacini and cysts containing

eosinophilic, periodic acid Schiff (PAS) positive secretions. Tumor cells have pale granular or vacuolated cytoplasm, low nuclear grade and are estrogen receptor (ER) negative. SBC has a favorable prognosis in children and adolescents, although it may recur locally or even metastasize, particularly in older women<sup>1,2</sup>.

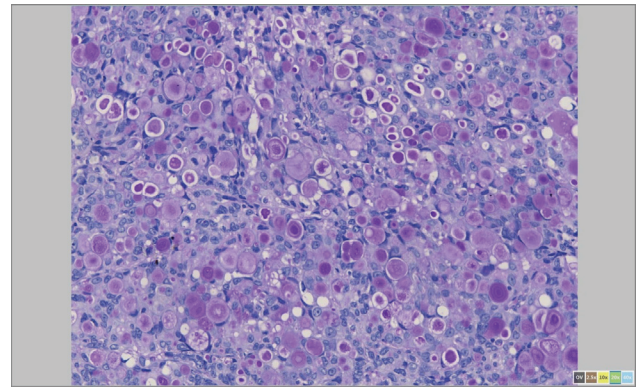
### Case report

A 67-year-old female patient presented with mass of the right breast since one month. After physical and routine laboratory examination, mammography was done. It showed presence of shadow in down right lateral area of the right breast measured 2 × 1.8 cm. Core needle biopsy was performed and histopathological (HP) examination confirmed an invasive carcinoma, probably of no special type. After an adequate preoperative preparation, operation was done – quadrantectomy with sentinel lymph node (SN) biopsy. Gross examination revealed presence of lobulated node diameter of 1.9 × 1.5 cm which was 1.5 cm to the closest resection margin. On the routine hematoxylin and eosin (HE) staining, the tumor showed pushing margin. The invasive tumor component was composed of solid areas of atypical tumor cells with granular cytoplasm as well as cells with signet-ring features. Also, there was a plenty of microcystic formations full of abundant eosinophilic material which was present in tumor cells as well (Figure 1).

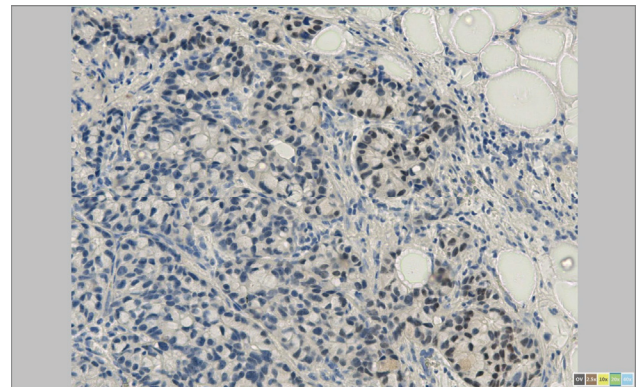


**Fig. 1 – Microcystic and solid areas of atypical tumor cells with granular cytoplasm and signet-ring features [hematoxylin and eosin (HE), 200×].**

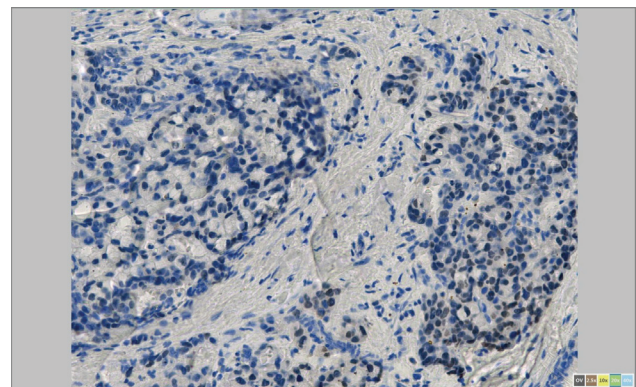
The nuclei of the tumor cells were small, round and low grade cytologically. The secretory material was clearly PAS and PAS-diastase positive (Figure 2). Immunohistochemically, ER and progesteron receptors (PR) showed weak positive reaction in 10% of tumor cells (Figure 3, 4), while human epidermal growth factor receptor-2 (HER-2) was without expression. SN and lymphovascular spaces were negative for the presence of the tumor. Postoperatively, the patient was treated with chemotherapy – 6 cycles of cyclophosphamide, methotrexate and fluorouracil (CMF), radiotherapy (60 Gray) and tamoxifen, also. After 5-year follow-up the patient had no signs of the disease.



**Fig. 2 – The secretory eosinophilic material [periodic acid Schiff (PAS), 200×].**



**Fig. 3 – Weak estrogen receptor positivity in single tumor cells [immunohistochemistry (IHC), 200×].**



**Fig. 4 – Weak progesteron receptor positivity in single tumor cells [immunohistochemistry (IHC), 200×].**

### Discussion

Secretory carcinoma is a very rare type of breast carcinoma, accounting for less than 0.1% of all infiltrating breast malignancies. It was first described in 1966 by McDivitt and Stewart<sup>3</sup> as juvenile carcinoma because they had 7 cases of this type of breast carcinoma without regional lymph node or distant metastases; patients ages ranged from 3 to 15 years, average 9 years SBC is most common under the age of 30, and it is the most common type of breast carcinoma in children. Although originally described in children, it is now known to occur in adults in both sexes, with male to female ratio 1:6. Reports suggest that the disease tends to be more

aggressive in males<sup>4</sup>. By 2003 there had been nearly 100 reported cases of SBC. Lamovec and Bracko<sup>5</sup> reported four cases of SBC among 7,038 breast carcinoma cases, and Botta et al.<sup>6</sup> found one case of SBC among 3,000 breast carcinoma cases. Li et al.<sup>7</sup> reported 15 SBC cases in their pathologic review of 10,000 breast carcinoma cases.

Clinically, the tumor usually presents as a mobile and palpable mass that is frequently subareolar but may occur in any part of the breast<sup>8-10</sup>. Tumor size ranges from 0.5 cm to 16 cm but it is usually between 1.5 cm and 3.0 cm, as in our case, and it tends to be larger in adults. Some authors consider that when larger than 2 cm it appears to have increased malignant potential<sup>11</sup>.

Radiographically, there are sparse descriptions of mammographic findings in the literature. SBC may be present as a discrete, lobulated, solitary mass with smooth or irregular borders which may mimic fibroadenoma. Because SBC occurs more frequently in young woman, mammography may have a diagnostic limitation because of the relatively high density of the breast tissue. A retroareolar dense mass is the usual finding in children as well as in male patients<sup>12-14</sup>.

Ultrastructurally, the tumor consists of large number of membrane-bound intracytoplasmic secretory vacuoles containing abundant secretory material<sup>4</sup>.

Grossly, it is often solitary, circumscribed, firm mass with grey, white or tan cut surface<sup>15</sup>.

Secretory carcinoma is histologically special type of breast carcinoma which has two main features – abundant PAS positive intracellular and extracellular secretions and a granular eosinophilic cell cytoplasm<sup>9</sup>. The tumor has predominantly pushing margins, occasionally with focal areas of infiltration.

Microscopically, this tumor is composed of polygonal cells with vacuolated pale-pink cytoplasm, granular eosinophilic or amphophilic cytoplasm and there is characteristic presence of large amounts of intracellular and extracellular secretory material which is variably reactive for mucin and PAS. Nuclei are small, round, bland with minimal atypia. Mitotic activity is low. Tumor cells are typically arranged into papillary, microcystic and glandular structures<sup>4, 15</sup>. Hyalinized fibrous tissue is frequently identified centrally. Sometimes, tumor cells may show prominent granular eosinophilic cytoplasm focally and higher nuclear grade, resembling apocrine carcinoma morphology<sup>16</sup>. Immunohistochemically, strong positive staining has been reported for S-100 protein, as well as  $\alpha$ -lactalbumin and polyclonal carcinoembryonic antigen (CEA). No reactivity was observed for gross cystic disease fluid protein-15 (GCDFP-15). It has been reported that secretory carcinoma is negative for ER, PR and HER2, so called “triple negative”<sup>2, 17</sup>. Recent studies suggest that SBC express basal-cell markers, including cytokeratins 5/6,

14 and 17, c-Kit (CD117), epidermal growth factor receptor and vimentin<sup>17-19</sup>. Also, there is consistently immunopositivity for S100 and  $\alpha$ -lactalbumin<sup>5</sup> and for E-cadherin as expected in any subtype of ductal carcinoma. Proliferation index by MIB-1 ranges from 1% to 34%<sup>20</sup>.

Cytogenetically, human SBC has been associated with balanced translocation t<sup>12, 15</sup> – associated ETV6-NTRK3 gene fusion as a dominantly acting oncogene in 12 of 13 cases<sup>21</sup> and it is the first demonstration of a balanced translocation in breast cancer. Also, some authors demonstrated that SBC with the ETV6-NTRK3 gene fusion belongs to the phenotypic spectrum of basal-like breast carcinomas<sup>17</sup>.

Locoregional recurrence is rare, but some authors have reported that there is an increased rate of recurrence in those cases treated by local resection<sup>11</sup>. Rosen and Cranor<sup>22</sup> have suggested that local recurrence is related to incomplete resection of the tumor. Tavassoli and Norris<sup>9</sup> have reported that metastasis to axillary nodes is not shown in tumors smaller than 2 cm in diameter. Distant metastases are extremely rare, and only four cases have been reported<sup>10</sup>.

Because of scarcity of reported cases, there is no published guidelines on the disease management. Surgical excision is the primary mode of the treatment for SBC with SN examination. In children, local excision with SN mapping is the preferred initial treatment with preservation of prepubertal breast tissue. In adults, because of the increased rate of recurrence, a simple mastectomy is recommended. Modified radical mastectomy has been favoured by some authors in cases with tumor size greater than 2 cm and poor gross circumscription<sup>11</sup>. Although there is no evidence to support, adjuvant chemotherapy and radiation have been tried for adults<sup>10</sup>. Recurrences may develop 6 and 8 years following both local excision and modified mastectomy, respectively. Distant metastases are extremely rare and were usually reported even after 20 years of a surgical treatment, so it is desirable to follow patients at least 20 years<sup>23</sup>.

## Conclusion

Secretory breast cancer is a rare subtype of invasive breast carcinoma with wide age range of occurrence and good prognosis despite its triple-negative immunophenotype. The histopathological diagnosis of this tumor is quite challenging although there is the specific morphologic pattern. SBC belongs to the phenotypic spectrum of basal-like breast carcinomas. Although the therapeutic management is non-consensual for this breast cancer special type, surgery is considered the mainstay of treatment as well as the adjuvant chemotherapy and radiation. Future molecular analyses may help for making targeted therapy for this disease.

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IN MEMORIAM

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**General-major u penziji  
primarijus dr BRANISLAV-BACKO POPOVIĆ  
(1931–2020)**

U Beogradu je 27. aprila 2020. godine umro general-major u penziji, primarijus dr Branimir Popović.

Rođen 1931. godine u selu Prekodolcu kod Vladičinog Hana. Završio je osnovnu školu u Vladičinom Hanu, gimnaziju u Vranju (1951), Medicinski fakultet (1959) i Školu rezervnih sanitetskih oficira (1960) u Beogradu, specijalizaciju opšte medicine u Školi narodnog zdravlja „Dr Andrija Štampar“ i u Vojnoj bolnici u Zagrebu (1967), a najvišu vojnu školu Jugoslovenske narodne armije, Školu narodne odbrane, u Beogradu 1982. godine.

U toku profesionalne oficirske i lekarske karijere obavljao je visoke i odgovorne dužnosti u sanitetskoj službi Jugoslovenske narodne armije (JNA): bio je referent sanitetske službe i upravnik ambulante u 166. gardijskom puku u Lukavici kod Sarajeva (1961–1964); nastavnik u Školi rezervnih oficira sanitetske službe u Beogradu (1965–1972); referent, načelnik odeljenja, zamenik načelnika, a u periodu od 1986. do 1988. godine i načelnik Sanitetske uprave Saveznog sekretarijata za narodnu odbranu, koja je bila najviši upravni organ sanitetske službe JNA. Od 1989. do 1992. godine bio je predsednik Glavne vojno-lekarske komisije JNA i sa te dužnosti je penzionisan 1992. godine u činu general-majora.

Na svakoj od navedenih dužnosti postizao je zavidne rezultate: kao trupni lekar najviše vremena je provodio u

kasarni i na logorskoj prostoriji brinući o zdravlju vojnika i oficira; kao nastavnik u Sanitetskoj oficirskoj školi prenosio je teorijska i praktična znanja iz sanitetske taktike lekarima, budućim sanitetskim oficirima i nosiocima rada u sanitetu JNA; pre postavljenja na dužnost načelnika Sanitetske uprave JNA bavio se planiranjem i organizacijom svih vidova školovanja i stručnog usavršavanja lekara i medicinskih tehničara u vojnom zdravstvenom sistemu; u periodu od 1986. do 1989. godine, u vreme kada je bio načelnik Sanitetske uprave JNA, rukovodio je radom celokupnog vojnog saniteta, od trupnih ambulanti kao primarnog nivoa zdravstvene zaštite, preko vojnih bolnica i vojnomedicinskih centara koji su obezbeđivali sekundarni nivo zdravstvene zaštite, do Vojnomedicinske akademije (VMA) kao najviše stručne, naučne i nastavne medicinske ustanove JNA. Dao je značajan doprinos unutrašnjoj organizaciji vojnih zdravstvenih ustanova, donošenju pravnih propisa koji regulišu rad saniteta, definisanju doktrine integrisane zdravstvene zaštite vojnog i civilnog zdravstva u vanrednim situacijama i ratu, kao i ulozi zdravstvene službe u opštenarodnom odbrambenom ratu. Uspešnom saradnjom sa sanitetskim službama više stranih armija doprineo je međunarodnom ugledu našeg saniteta.

Posle penzionisanja, od 1992. do kraja 1995. godine, general Branimir Popović je dobrovoljno učestvovao u radu

saniteta na ratištima na teritoriji Republike Srpske Krajine i Republike Srpske, znatno doprinoseći boljoj organizaciji i efikasnijem radu sanitetskih jedinica i ustanova na tim prostorima.

General-major dr Branislav-Backo Popović je bio predsednik Saveza udruženja boraca Narodnooslobodilačkog rata (SUBNOR) za grad Beograd (2007–2014) i potpredsednik SUBNOR-a Republike Srbije od 2007. godine do smrti. Kao dugogodišnji visoki rukovodilac SUBNOR-a dao je značajan doprinos formiranju i radu opštinskih organizacija na teritoriji cele Srbije, a svojim radom je širio i afirmisao slobodarski i antifašistički duh i veru u bolji i napredniji svet, smatrajući da su sloboda i socijalna pravda najveće bogatstvo i da zbog toga nemaju cenu.

Pored redovnih radnih obaveza bavio se i publicističkim i istraživačkim radom. Objavio je oko 180 stručnih radova iz oblasti organizacije zdravstvene zaštite i sanitetske službe u miru, vanrednim situacijama i ratu, ali i iz vojne medicine, istorije sanitetske službe i gerontologije. Autor je ili koautor u deset monografija i preko 50 neautorizovanih radova u formi studija, elaborata, stručnih pravila, priručnika i propisa sa vojnom tematikom. Napisao je sa grupom autora značajno delo „Vojni sanitet u srpskom narodu“, koje je imalo dva izdanja (1998. i 2002 godine). Urednik je pet monografija o srpskom vojnom sanitetu u Balkanskim ratovima i Prvom svetskom ratu i koautor monografije o partizanskom sanitetu u Drugom svetskom ratu. Pre pola godine izašla je iz štampe njegova autobiografija pod naslovom „Život jednog vojnog lekara“, koja može da bude primer i putokaz sadašnjim i budućim generacijama lekara kako se obavlja časna i odgovorna dužnost vojnog lekara.

Učestvovao je u organizaciji 20 medicinskih kongresa i simpozijuma. Bio je član uredništva vojno-teorijskih i stručnih medicinskih časopisa („Vojnosanitetski pregled“, „Vojno delo“ i „Pozadina“). Učestvovao je u realizaciji 13 naučno-istraživačkih projekata iz oblasti organizacije vojne medicine, zdravstvene zaštite i edukacije medicinskih kadrova.

Član je Srpskog lekarskog društva od 1967. godine. Ima zvanje primarijusa od 1991. godine. Počasni je član Akademije medicinskih nauka Srpskog lekarskog društva od 2000. godine. Dobitnik je „Nagrade za životno delo“ Srpskog lekarskog društva 2015. godine i nagrade „Dr Vladan Đorđević“ 2017. godine, koju dodeljuje Sekcija za istoriju medicine Srpskog lekarskog društva za životno delo i trajan doprinos proučavanju istorije srpske medicine. Bio je član Predsedništva Saveza lekarskih društava Jugoslavije (1986–1989).

U privatnom životu bio je posvećen porodici. Supruga Emilija, ćerka Suzana i sin Zoran, kao i članovi njihovih porodica, činili su topli dom u kojem je nalazio mir i zadovoljstvo, kako u trenucima sreće, tako i u iskušenjima.

General dr Branislav Popović je bio svestrana ličnost, ostvaren ne samo kao lekar i oficir, nego i u privatnom životu i mnogim oblastima društvene i profesionalne delatnosti. Posedovao je osobine vrhunskog lekara i oficira – profesionalca: lično i akademsko poštenje, kompetentnost, inicijativnost, sistematičnost, pronicljivost, upornost. Sve je procenjivao realno, sa dozom kritičnosti vrhunskog intelektualca: i svet oko sebe, i ljude, i društvena kretanja, i sopstvene rezultate, i svakodnevne događaje. Na osnovu realnih procena, postavljao je sebi i drugima ostvarive zahteve i ciljeve, vršio je temeljne i sistematične pripreme, pratio svaku fazu bilo kog posla kojim se bavio. Jednako dobro je zapažao greške i napretke, uspehe i nedostatke, i uvek nalazio načine za dodatne motive, pomerao granice ostvarivog u nekim teškim situacijama.

General dr Branislav Popović je svojim životom i radom dao trajni doprinos sanitetskoj službi, celokupnoj srpskoj medicini, istoriji medicine i temeljnim vrednostima društva svog vremena. Zbog toga zaslužuje našu veliku zahvalnost i poštovanje!

doc. dr Veljko Todorović, brig. general u penziji

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U VSP-u se objavljuju **uvodnici, originalni članci, prethodna ili kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme, metaanalize, kazuistika, seminar praktičnog lekara, članci iz istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

Rukopis se piše sa proredom 1,5 sa levom marginom od 4 cm. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office (Excel, Word Graph)**. Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje autoru određenom za korespondenciju na konačnu saglasnost.

### Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst rada, zahvalnost** (po želji), literatura, prilozi.

#### 1. Naslovna strana

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

b) Ispisuju se puna imena i prezimena autora sa oznakama redom: \*, †, ‡, §, ||, ¶, \*\*, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespondenciju.

#### 2. Apstrakt i ključne reči

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure – **Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi – **Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz**

**bolesnika i Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

### 3. Tekst članka

Tekst sadrži sledeća poglavlja: **uvod, metode, rezultate i diskusiju**. **Uvod**. Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

**Metode**. Jasno opisati izbor metoda posmatranja ili eksperimentalnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

**Rezultate** prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

### Literatura

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

### Primeri referenci:

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

### Tabele

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

### Ilustracije

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

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

### Skraćenice i akronimi

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

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

**Detaljno uputstvo može se dobiti u redakciji ili na sajtu:**  
[www.vma.mod.gov.rs/vsp](http://www.vma.mod.gov.rs/vsp)