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ORIGINAL ARTICLES / ORIGINALNI RADOVI

World Heart Day - 29th September 2022

World Heart Day (WHD) is celebrated every year on 29 September. The theme of WHD 2022 is 'USE HEART FOR EVERY HEART'. The aim of WHD is to inform people around the globe that cardiovascular diseases (CVD), including heart disease and stroke, is the world's leading cause of death, claiming 17.9 million lives each year, which is 32% of all global deaths. It aims to educate people that by controlling risk factors such as tobacco use, unhealthy diet, and physical inactivity, at least 80% of premature deaths from heart disease and stroke could be avoided.

Svetski dan srca - 29. septembar 2022.

Svetski dan srca (SDS) obeležava se svake godine 29. septembra. Tema SDS 2022. godine je 'KORISTI SRCE ZA SVAKO SRCE'. Cilj SDS je da širi svest ljudi da su kardiovaskularne bolesti (KVB), uključujući srčane bolesti i moždani udar, vodeći uzrok smrti ljudi u svetu, odgovorne za gubitak 17.9 miliona života svake godine, što je 32% svih smrtnih slučajeva na globalnom nivou. Ovaj Dan ima za cilj da edukuje ljude da se kontrolom faktora rizika kao što su upotreba duvana, nezdrava ishrana i fizička neaktivnost, može izbeći najmanje 80% fatalnih ishoda zbog bolesti srca i moždanog udara.

ORIGINAL ARTICLES

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Significance of initial clinical laboratory parameters as prognostic factors in patients with COVID-19

Značaj inicijalnih kliničko-laboratorijskih parametara kao prognostičkih faktora kod bolesnika sa COVID-19

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University of Kragujevac, Faculty of Medical Sciences, *Department of Infectious Diseases, ‖Department of Pharmacology and Toxicology, Kragujevac, Serbia; University Clinical Center Kragujevac, †Clinic for Infectious Diseases, ‡Department of Radiological Diagnostics, §Department of Clinical Pharmacology, Kragujevac, Serbia

Abstract

Background/Aim. Coronavirus disease 2019 (COVID-19) is a predominantly respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The aim of this study was to determine whether there were parameters that could predict the development of a severe clinical picture and fatal outcomes in COVID-19 patients. **Methods.** The study involved 632 patients treated at the Clinic for Infectious Diseases, University Clinical Center Kragujevac, from June 2020 to February 2021. All patients were divided into two groups according to the need for oxygen therapy (Sat 0_2 < 94 %). **Results.** Our results showed that high body mass index (BMI) was singled out as a risk factor for the development of a severe clinical picture (BMI, $OR_{adjusted}$ = 1.263; 95% CI = 1.117 – 1.427; p < 0.001). Prothrombin time ($OR_{adjusted}$ = 1.170; 95% CI = 1.004 - 1.364; p = 0.045), as well as low albumin values ($OR_{adjusted}$ = 0.878; 95% CI = 0.804 - 0.958; p = 0.003), had a predictive significance for the development of a severe clinical picture. Factors that were of predictive importance in patients with fatal outcomes were C-reactive protein (CRP) ($OR_{adjusted}$ = 1.010; 95% CI = 1.001 – 1.019; p = 0.031), lactate dehydrogenase (LDH) ($OR_{adjusted}$ = 1.004; 95% CI = 1.001 – 1.006; p = 0.002), and X-ray of the lungs ($OR_{adjusted}$ = 1.394; 95% CI = 1.170 – 1.661; p < 0.001). **Conclusion.** The study showed that routine, clinical laboratory parameters can be important in the early detection of patients with a potentially severe clinical picture and fatal outcomes. In patients with a mild clinical picture, CRP, LDH, ferritin, and serum albumin levels may timely indicate disease progression. Monitoring these parameters is of essential importance for the timely clinical assessment of patients with COVID-19 and, thus, the prompt application of adequate therapeutic protocols in the treatment of these patients.

Key words:
biomarkers; body mass index; covid-19; death; disease progression; hematologic tests; prognosis; sars-cov-2; severity of illness index; treatment outcome.

Apstrakt

Uvod/Cilj. Bolest koronavirus 2019 (COVID-19) je dominantno respiratorna bolest koju izaziva *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2). Cilj rada bio je da se utvrdi postojanje parametara koji bi mogli da predvide razvoj teške kliničke slike i smrtni ishod kod obolelih od COVID-19. **Metode.** Istraživanjem su bila obuhvaćena 632 bolesnika lečena na Klinici za infektivne bolesti Univerzitetskog kliničkog centra Kragujevac, u periodu od juna 2020. godine do februara 2021. godine. Svi bolesnici su bili podeljeni u dve grupe na osnovu potrebe za kiseoničnom terapijom (Sat 0_2 < 94%) **Rezultati.** Visok indeks telesne mase izdvojio se kao faktor rizika od razvoja teške kliničke slike ($OR_{adjusted}$ = 1,263; 95% CI = 1,117 - 1,427; p < 0,001). Prognostički značaj za razvoj teške kliničke slike imalo je i protrombinsko vreme ($OR_{adjusted}$ = 1,170; 95% CI = 1,004 – 1,364; p = 0,045), kao i niske vrednosti albumina ($OR_{adjusted}$ = 0,878; 95% CI = 0,804 – 0,958; p = 0,003). Faktori koji su imali prognostički značaj kod bolesnika čija se bolest završila smrtnim ishodom bili su C-reaktivni protein (CRP) ($OR_{adjusted}$ = 1,010; 95% CI = 1,001 – 1,019; p = 0,031), laktat dehidrogenaza (LDH) ($OR_{adjusted}$ = 1,004; 95% CI = 1,001 – 1,006; p = 0,002) i radiografski nalaz pluća ($OR_{adjusted}$ = 1,394; 95% CI = 1,170 – 1,661; p < 0,001). **Zaključak.** Istraživanje je pokazalo da rutinski, kliničko-laboratorijski parametri mogu imati značaj u ranom otkrivanju obolelih sa potencijalno teškom

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kliničkom slikom i smrtnim ishodom. Kod obolelih sa lakom kliničkom slikom, CRP, LDH, feritin i nivo albumina u serumu mogu blagovremeno ukazati na progresiju bolesti. Praćenje ovih parametara je od velikog značaja za pravovremenu kliničku procenu stanja obolelih od COVID-19, a samim tim i pravovremenu primenu adekvatnih terapijskih protokola za lečenje tih bolesnika.

Ključne reči:
biološki pokazatelji; telesna masa, indeks; covid-19; smrt; bolest, progresija; hematološki testovi; prognoza; sars-cov-2; bolest, indeks težine; lečenje, ishod.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and currently presents as a dominant respiratory disease worldwide [1]. SARS-CoV-2 is considered more contagious than severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) [2]. COVID-19 is manifested by a variety of symptoms, including fever, exhaustion, loss of sense of smell and taste, croup, runny nose, cough, shortness of breath, etc. The clinical presentation is different and may include patients with asymptomatic infection, upper respiratory tract infection, and viral pneumonia [3]. Early detection of asymptomatic patients is very important in preventing the spread of the infection [4, 5]. Asymptomatic patients, by the end of clinical follow-up in most cases, remain asymptomatic with a good prognosis, without developing complications [6]. Patients with COVID-19 with pneumonia develop a secondary bacterial infection to a lesser extent than patients with influenza [7]. Severe COVID-19 occurs as a result of a systemic inflammatory response (SIRS) when other organ systems, such as the cardiovascular system, kidneys, and liver, are damaged [8, 9]. The mechanism of damage is most likely related to the expression of angiotensin-converting enzyme (ACE)-2 receptors on other organ systems, in addition to the lungs [7]. Therefore, it is extremely important to timely identify patients with risk factors of developing severe clinical manifestations, primarily pneumonia, as well as other manifestations. Patients with risk factors for the progression of the severe clinical picture should be candidates for the inclusion of adequate therapeutic protocols. The fundamental question to be asked is whether routine analyses and some clinical characteristics can exhibit predictive significance for the development of a severe clinical outcome.

The aim of the study was to determine the variables that affect the progression of the severe clinical picture of COVID-19, as well as to determine which variables may contribute to the fatal outcome of COVID-19. Moreover, one of the goals was to determine whether there were patients who switched from mild to severe patients during clinical follow-up and whether certain variables were isolated in such patients.

Methods

The study involved 632 patients treated at the Clinic for Infectious Diseases, University Clinical Center (UCC) Kragujevac in Serbia, from June 2020 to February 2021. UCC Kragujevac encompasses a region to which two million people gravitate. The study included patients who had COVID-19 confirmed by nasopharyngeal swabs [polymerase chain reaction (PCR) test or antigen (Ag) test].

All patients were divided into two groups according to the need for oxygen (O_2) therapy [with O_2 saturation (Sat O_2) < 94%]. The first group consisted of patients with Sat O_2 < 94% and the second group consisted of patients with Sat O_2 > 94%. The application of O_2 therapy implies different types of O_2 therapy depending on the degree of acute respiratory insufficiency [O_2, high flow O_2 nasal therapy (HFNO), non-invasive ventilation (NIV), mechanical ventilation (MV)]. Patients who did not use O_2 support (Sat O_2 > 94%) belonged to the group of patients with a mild form of the illness. These patients were treated in the early stages of a pandemic when hospitalization was mandatory for all people with SARS-CoV-2 positive tests. Patients aged 18 or above were included in the study. Body mass index (BMI) values were measured in all patients; therefore, values > 25 kg/m^2 were defined as overweight, while BMI values over 30 kg/m^2 were defined as obesity.

Upon admission to the hospital, all patients underwent initial laboratory analyses in the first 24 hrs, which included basic hematological analyses (complete blood count with leukocyte formula), biochemical analyses [urea, creatinine, potassium (K), sodium (Na), glycemia, C-reactive protein (CRP), lactate dehydrogenase (LDH), procalcitonin (PCR), ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma GT, creatine kinase (CK), CK–MB, troponin, iron (Fe)]. Coagulation status [international normalized ratio (INR), activated partial thromboplastin time (aPTT), D-dimer, fibrinogen] of all patients was measured as well.

All laboratory analyses were performed in the Laboratory Diagnostics Service of the UCC Kragujevac. As a model for the analysis of radiographic lung findings, we used the scoring proposed by Italian radiologists from Brescia in May 2020 [10]. The lungs were divided into six zones: Line A – in the projection of the lower wall of the aortic arch; Line B – in the projection of the lower wall of the right pulmonary vein. The second step was to score each of the six zones according to the changes in the lungs: 0 – no changes in lungs, 1 – interstitial infiltrate, 2 – alveolar infiltrates, and 3 – interstitial and alveolar infiltrate. An X-ray found changes in the lungs were described within the range of severity from 1 to 18.

The study was approved by the Ethics Committee of UCC Kragujevac (01/20-498, on May 5, 2020). All collected data were organized into an electronic database.

Table 1

Variables monitored in patients with COVID-19 with severe and mild clinical pictures

Variable	Patients with severe clinical picture (n = 312)	Patients with mild clinical picture (n = 320)	Significance
Age (years), mean ± SD	61.64 ± 13.08	48.41 ± 15.18	t = 11.725 p < 0.001
Radiology score, mean ± SD	10.23 ± 4.11	2.61 ± 3.19	t = -25.894 p < 0.001
DM type 2, n (%)	100 (32.8)	25 (8.1)	χ^2 = 50.024 p < 0.001
HTA, n (%)	175 (57.0)	61 (27.6)	χ^2 = 81.042 p < 0.001
Asthma/COPD, n (%)	18 (5.9)	8 (2.6)	χ^2 = 4.155 p = 0.042
Body mass index (kg/m^2), mean ± SD	28.81 ± 4.33	24.84 ± 3.97	t = -11.957 p < 0.001
Leukocytes (x10^9/L), mean ± SD	7.40 ± 3.62	5.99 ± 2.80	t = 5.436 p < 0.001
Lymphocytes (x10^9/L), mean ± SD	0.93 ± 1.46	1.46 ± 1.06	t = -7.101 p < 0.001
Platelets (x10^9/L), mean ± SD	220.95 ± 104.07	223.20 ± 86.18	t = -0.293 p = 0.770
Glucose (mmol/L), mean ± SD	8.43±4.83	6.10 ± 2.30	t = 7.537 p < 0.001
Urea (mmol/L), mean ± SD	7.74 ± 4.81	5.19 ± 2.30	t = -8.391 p < 0.001
Creatinine (μmol/L), mean ± SD	106.16 ± 108.91	88.08 ± 56.72	t = -2.583 p = 0.010
Potassium (mmol/L), mean ± SD	3.99 ± 0.54	4.15 ± 0.42	t = -4.208 p < 0.001
Sodium (mmol/L), mean ± SD	137.79 ± 4.10	138.63 ± 2.85	t = -2.837 p = 0.005
Albumins (g/L), mean ± SD	34.79 ± 4.87	41.18 ± 4.28	t = -17.327 p < 0.001
CRP (mg/L), mean ± SD	114.67 ± 87.23	28.19 ± 49.35	t = -15.227 p < 0.001
PCT (ng/mL), mean ± SD	0.54 ± 2.68	0.08 ± 0.35	t = 3.041 p = 0.002
AST (IU/L), mean ± SD	59.72 ± 94.68	38.25 ± 49.94	t = 3.507 p < 0.001
ALT (IU/L), mean ± SD	56.73 ± 81.12	42.93 ± 63.38	t = -2.343 p = 0.019
Bilirubin total (μmol/L), mean ± SD	10.92 ± 4.75	10.02 ± 5.56	t = 2.130 p = 0.034
GGT (IU/L), mean ± SD	68.35 ± 89.51	43.89 ± 54.99	t = 4.222 p < 0.001
CK-MB (U/L), mean ± SD	15.52 ± 14.50	11.58 ± 4.93	t = -4.508 p < 0.001
CK (U/L), mean ± SD	227.81 ± 397.13	127.87 ± 163.92	t = -4.082 p < 0.001
LDH (U/L), mean ± SD	755.84 ± 439.27	433.27 ± 185.53	t = -11.770 p < 0.001
D-dimer (μg/mL FEU), mean ± SD	2.38 ± 7.99	0.85 ± 3.20	t = -3.096 p = 0.002
Fibrinogen (g/L), mean ± SD	6.03 ± 1.79	4.28 ± 1.86	t = 11.219 p < 0.001
PT (sec), mean ± SD	15.01 ± 6.01	12.15 ± 2.83	t = -7.051 p < 0.001
INR, mean ± SD	1.21 ± 0.48	1.07 ± 0.26	t = -4.027 p < 0.001
Troponin (ng/mL), mean ± SD	0.05 ± 0.30	0.01 ± 0.02	t = 2.382 p = 0.018
Ferritin (μg/L), mean ± SD	977.86 ± 901.19	353.22 ± 435.26	t = -10.757 p < 0.001
Fe (μmol/L), mean ± SD	5.25 ± 4.31	10.17 ± 11.54	t = -10.757 p < 0.001

DM – diabetes mellitus; HTA – hypertensio arterialis; COPD – chronic obstructive pulmonary disease; CRP – C-reactive protein; PCT – procalcitonin; AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT – gamma-glutamyl transferase; CK – creatine kinase; CK-MB – creatine kinase-MB fraction; LDH – lactate dehydrogenase; FEU – fibrinogen equivalent units; PT – prothrombin time; INR – international normalized ratio; SD – standard deviation.

Statistical analysis

The data were described by descriptive statistics, using measures of central tendency (mean), variability (standard deviation from the mean), and relative numbers. After testing for normality of the data distribution with the Kolmogorov-Smirnov test, the significance of the difference in values of continuous variables between the study groups defined by categorical variables was tested by Student's *t*-test for independent samples. The significance of the difference in categorical variables between the study groups was tested by the Chi-squared test. The differences were considered significant if the probability of the null hypothesis was ≤ 0.05. Associations between putative risk factors and the study outcome were tested by the multivariate logistic regression model. All calculations were performed by the SPSS (Statistical Package for Social Science for Windows) software, version 18.

Results

A total of 632 patients, who were hospitalized for COVID-19, participated in the study. Among them, 312 (49.4%) were patients with severe clinical pictures, while 320 (50.6%) were patients with mild clinical pictures. Patients with severe clinical pictures required some types of O_2 therapy (O_2, HFNO, NIV, MV). Fatal outcomes were recorded in 57 (11.08%) patients. On O_2 were 177 (56.7%) patients with severe clinical pictures, while on HFNO were 80 (25.6%) patients. There were 55 (17.6%) patients on MV with the most severe clinical status, of which 4 (7.27%) had a favorable outcome. On hospital admission, the initial O_2 was 6 L/min, while the average number of days on O_2 therapy was 11 days. The average duration of HFNO in patients was 6 days, while the average duration of MV was 11 days.

The average age of patients with a severe clinical picture was 61.64 ± 13.08 years, while of patients with mild clinical pictures was 48.41 ± 15.18 years. There was a higher percentage of patients with comorbidities in the group of patients with severe clinical pictures compared to those with mild clinical pictures. Hypertension was recorded in 175 (57.0%) patients with severe clinical pictures, while diabetes mellitus was recorded in 100 (32.8%) patients with severe clinical pictures. The number of patients with asthma/chronic obstructive pulmonary disease (COPD) during the second and third waves of COVID-19, in the group of mild 8 (2.6%) and severe patients 18 (5.9%), was significantly lower than the number of patients with cardiovascular diseases and diabetes mellitus.

The results of the univariate analyses of the possible risk factors for the development of a severe clinical picture are shown in Table 1.

In the group of patients with severe clinical pictures, a significant number of patients with higher BMI values was observed, and their average score of X-ray findings of the lungs was higher than in the group of patients with mild clinical pictures.

The results of hematological and biochemical parameters are shown in Table 1.

According to the multivariate logistic regression, factors associated with the development of a severe clinical picture of COVID-19 were BMI, prothrombin time, and RDG score (chest X-ray scoring system [10]), while blood albumin level was a protective factor (Table 2).

In this study, we analyzed risk factors for death in a group of severe patients. Binary logistic regression showed that the decisive parameter associated with fatal outcomes was the use of the mechanical ventilation (OR = 8,259.43; 95% CI = 219.60−310,646.90; $p < 0.001$), while the number of leukocytes in the blood was singled out as a protective factor in a group of severe patients (OR = 0.763; 95% CI = 0.602−0.968, $p = 0.026$).

Group of patients with fatal outcomes

Fatal outcomes occurred in 57 patients out of a total of 632 patients who participated in the study. The average age in the group of patients with fatal outcomes was 68 years (67.61 ± 10.93), unlike patients who survived COVID-19, where the average age was 53 years (53.59 ± 15.51) (Table 3).

The laboratory data of patients with fatal outcomes vs. the ones who survived COVID-19 are shown in Table 3.

In the group of patients with fatal outcomes, there were higher values of BMI, and a higher number of such patients had comorbidities (hypertension and diabetes mellitus) compared to the group of patients who survived.

As expected, radiological findings in the lungs had a high score in the group of patients with fatal outcomes, 13.04 ± 4.13, while the score in the group of patients that survived was 5.76 ± 4.94.

Blood count analysis indicates that significantly higher leukocyte counts and significantly lower lymphocyte counts were observed in patients with fatal outcomes ($t = 4.414$, $p < 0.001$ and $t = -2.179$, $p = 0.030$, respectively), while platelet

Table 2

Multivariate analysis in COVID-19 patients with severe and mild clinical pictures

Variable	AOR	95% CI	*p*-value
BMI	1.263	1.117–1.427	< 0.001
Albumins	0.878	0.804–0.958	0.003
PLT	1.170	1.004–1.364	0.045
RDG score	1.435	1.272–1.619	< 0.001

AOR – adjusted odds ratio; CI – confidence interval; BMI – body mass index; PLT – platelets; RDG score – chest X-ray scoring system for hospitalized patients with COVID-19 pneumonia [10].

Table 3

Variables measured in the group of patients with fatal outcome and patients who survived COVID-19

Variable	Patients with fatal outcome (n = 57)	Patients who survived COVID-19 (n = 570)	Significance
Age (years), mean ± SD	67.61 ± 10.93	53.59 ± 15.51	$t = 6.662$ $p < 0.001$
Radiology, mean ± SD	13.04 ± 4.13	5.76 ± 4.94	$t = 10.753$ $p < 0.001$
DM type 2, n (%)	24 (45.3)	101 (18)	$\chi^2 = 22.309$ $p < 0.001$
HTA, n (%)	37 (68.5)	205 (36.4)	$\chi^2 = 21.307$ $p < 0.001$
Asthma/COPD, n (%)	1 (1.8)	25 (4.5)	$\chi^2 = 0.877$ $p = 0.349$
Body mass index (kg/m^2), mean ± SD	28.95 ± 4.56	26.58 ± 4.57	$t = 3.701$ $p < 0.001$
Leukocytes (x10^9/L), mean ± SD	8.53 ± 4.23	6.52 ± 3.16	$t = 4.414$ $p < 0.001$
Lymphocytes (x10^9/L), mean ± SD	0.92 ± 1.56	1.22 ± 0.89	$t = -2.179$ $p = 0.030$
Platelets (x10^9/L), mean ± SD	212.23 ± 92.66	223.32 ± 95.91	$t = -0.835$ $p = 0.404$
Glucose (mmol/L), mean ± SD	10.37 ± 6.95	6.95 ± 3.40	$t = 6.364$ $p < 0.001$
Urea (mmol/L), mean ± SD	10.36 ± 6.22	6.06 ± 3.44	$t = 8.185$ $p < 0.001$
Creatinine (μmol/L), mean ± SD	141.01 ± 196.30	92.61 ± 65.54	$t = 4.044$ $p < 0.001$
Potassium (mmol/L), mean ± SD	4.04 ± 0.60	4.07 ± 0.48	$t = -0.481$ $p = 0.630$
Sodium (mmol/L), mean ± SD	138.47 ± 4.97	138.19 ± 3.49	$t = 0.533$ $p = 0.594$
Albumins (g/L), mean ± SD	32.87 ± 4.71	38.53 ± 5.64	$t = -7.507$ $p < 0.001$
CRP (mg/L), mean ± SD	144.55 ± 103.61	64.08 ± 77.06	$t = 7.253$ $p < 0.001$
PCT (ng/mL), mean ± SD	0.60 ± 0.85	0.2807 ± 1.99	$t = 1.204$ $p = 0.229$
AST (IU/L), mean ± SD	93.00 ± 207.01	44.54 ± 43.51	$t = 4.637$ $p < 0.001$
ALT (IU/L), mean ± SD	72.93 ± 161.91	47.56 ± 56.38	$t = 2.508$ $p = 0.012$
Bilirubin total (μmol/L), mean ± SD	11.74 ± 6.15	10.34 ± 5.06	$t = 1.946$ $p = 0.052$
GGT (IU/L), mean ± SD	84.37 ± 134.06	53.79 ± 65.95	$t = 2.936$ $p = 0.003$
CK-MB (U/L), mean ± SD	19.89 ± 16.03	12.89 ±10.13	$t = 4.659$ $p < 0.001$
CK (U/L), mean ± SD	329.91 ± 613.48	162.19 ± 252.68	$t = 3971$ $p < 0.001$
LDH (U/L), mean ± SD	1063.01 ± 746.19	549.11 ± 272.55	$t = 10.721$ $p < 0.001$
D-dimer (μg/mL FEU), mean ± SD	2.35 ± 3.44	1.54 ± 6.33	$t = 0.939$ $p = 0.348$
Fibrinogen (g/L), mean ± SD	6.09 ± 1.93	5.05 ± 2.01	$t = 3.366$ $p = 0.001$
PT (sec), mean ± SD	15.39 ± 4.34	13.40 ± 4.93	$t = 2.788$ $p = 0.005$
INR (INR), mean ± SD	1.20 ± 0.37	1.13 ± 0.40	$t = 1.234$ $p = 0.218$
Troponin (ng/mL), mean ± SD	0.07 ± 0.18	0.03 ± 0.22	$t = 1.263$ $p = 0.207$
Ferritin (μg/L), mean ± SD	1163.95 ± 997.50	619.67 ± 731.84	$t = 5.064$ $p < 0.001$
Fe (μmol/L), mean ± SD	4.59 ± 3.41	7.88 ± 9.21	$t = -2.220$ $p = 0.027$

For abbreviations see under Table 1.

counts (t = -0.835, p = 0.404) showed no statistical significance.

Inflammatory parameters, such as CRP (t = 7.253, p < 0.001), showed statistical significance in the group of deceased patients compared to surviving patients, while values of PCT (t = 1.204, p = 0.229) did not show statistical significance between the two groups.

Ferritin and LDH values (t =10.721, p < 0.001) were higher in the deceased patients' group in relation to the group of surviving patients, while the values of D-dimers (t = 0.939, p = 0.348) did not show a statistically significant difference between the two groups. In addition, analysis of cardiac markers, such as troponin, did not show significance in fatal outcomes (t = 1.263, p = 0.207).

According to the multivariate logistic regression, factors associated with death outcome were CRP ($OR_{adjusted}$ = 1.010; 95% CI = 1.001 – 1.019; p = 0.031), LDH ($OR_{adjusted}$ = 1.004; 95% CI = 1.001 – 1.006; p = 0.002), and RDG score ($OR_{adjusted}$ = 1.394; 95% CI = 1.170 – 1.661; p < 0.001); K blood level was a protective factor ($OR_{adjusted}$ = 0.261; 95% CI =0 .073 – 0.930; p = 0.038) (Table 4).

Table 4

Multivariate analysis in the group of patients with fatal outcome and patients who survived COVID-19

Variable	AOR	95% CI	*p*
Potassium blood level	0.261	0.073–0.930	
CRP	1.010	1.001–1.019	0.031
LDH	1.004	1.001–1.006	0.002
RDG score	1.394	1.170–1.661	< 0.001

AOR – adjusted odds ratio; CI – confidence interval; CRP – C-reactive protein; LDH – lactate dehydrogenase; RDG score – chest X-ray scoring system for hospitalized patients with COVID-19 pneumonia [10].

Patients who switched from a group of patients with mild clinical pictures to a group of patients with severe clinical pictures during clinical follow-up

A total of 320 patients who belonged to the group of patients with light clinical pictures participated in the study; during the hospital treatment, the general condition worsened, which meant switching to the group of patients with heavy clinical pictures. Worsening of the general condition meant a drop in saturation below 93% and the urgent need for O_2 therapy. Among patients who experienced exacerbations, 7 (30%) patients had a BMI over 30, indicating obesity. Moreover, in 8 (43%) patients, cardiac diseases were registered, primarily hypertension.

When comparing different variables between the group of patients with mild symptoms (321) and those who switched to the group of patients in severe state (21) during clinical follow-up, the statistical significance between certain biochemical parameters and radiographic findings in the lungs was achieved. Decreased albumin (< 0.001), increased CRP (< 0.001), ferritin (< 0.001), and LDH (< 0.001) were reported in patients whose clinical picture worsened and progressed to the severe clinical picture group. Furthermore, patients whose condition worsened during follow-up initially had a worse radiographic score (< 0.001) compared to patients in the light patient group.

Discussion

Although COVID-19 has been present worldwide for more than a year, it remains largely unexplored in clinical work. The main aim of the study was to discover parameters that could help us in our daily work with COVID-19 patients and indicate the development of severe clinical manifestations. Most clinical laboratory parameters are routine analyses performed on all patients in the first 24 hrs of hospitalization, which significantly facilitated our research.

Obesity was singled out as a predictive factor for the occurrence of the severe clinical picture in our sample, which is certainly one of the important characteristics of patients with severe outcomes. Obtained results showed that BMI values in our sample were significantly higher than in the results of Chinese authors [11], while an extensive study conducted in the UK showed that BMI over 23 kg/m^2 posed a risk for severe COVID-19 [12]. Obesity adversely affects the prediction of COVID-19 because lung function is associated with decreased expiratory reserve volume, functional capacity, and respiratory compliance [13]. It is considered that chronic inflammation in obese patients adversely affects the course of the disease [14]. The progression of the severe clinical picture is also influenced by the fact that the receptor for the human ACE is, to a greater extent, expressed in adipose tissue than in the lungs [15]. For that reason, obese people express a large number of receptors for the SARS-CoV-2 virus, so they are more susceptible to infection. The number of registered patients with cardiovascular comorbidities and diabetes mellitus was much higher compared to the Barcelona cohort examined in 322 patients [16]. Although comorbidities, primarily hypertension, and diabetes mellitus, were more common in the group of patients with a severe clinical picture, they did not exhibit predictive significance in our study.

When we analyzed basic biochemical analyses that could affect the progression of the severe clinical pictures, reduced albumin values that could exhibit predictive significance were singled out. Hypoalbuminemia in COVID-19 is considered to occur not only due to hepatocellular damage but also as a result of systemic inflammation and increased capillary permeability, causing albumin to disappear into the interstitial

space [17]. In this way, hypoalbuminemia, as a consequence of acute phase protein synthesis, reflects the strength of the SIRS and, therefore, has predictive significance in COVID-19.

Liver damage is quite common in COVID-19. It is known that in addition to the lungs, other organ systems are affected as well. Therefore, elevated values of transaminases, which did not exhibit predictive significance for the progression of the severe clinical picture, were registered. Liver damage in COVID-19 occurs primarily due to the direct action of the virus since the ACE receptor is expressed throughout the liver and the biliary tract [18]. Liver damage is also affected by pre-existing conditions such as obesity, chronic liver infection, and cirrhosis of various etiologies. We should not forget the toxic effect of drugs, especially antiviral drugs and macrolides, which are most often administered in the treatment of COVID-19 patients [19].

The analysis of coagulation status singled out only the elevated value of PT as a significant predictive marker for the severe clinical picture, as demonstrated by the results of other authors [20, 21], although further research is more than necessary. It is known that PT is prolonged in the case of a decrease in coagulation factors, most often in liver damage, as well as in the use of various inhibitors [22]. On the other hand, coagulation parameters did not prove to exhibit predictive significance for fatal outcomes.

When analyzing biochemical parameters, our results showed that elevated CRP values, elevated LDH values, and hypokalemia exhibited predictive significance for fatal outcomes.

Other authors have already shown that elevated CRP values exhibit predictive significance for the fatal outcomes of COVID-19. CRP as a protein of the acute phase of protein is elevated in infection but also inflammation [23]. It is functionally linked to interleukin-6, which induces gene expression and CRP release from the liver [24].

LDH is an intracellular enzyme present in many cells, as well as in the lungs, which is why its release is expected in severe interstitial pneumonia, which can progress to acute respiratory distress syndrome (ARDS) and multi-organ dysfunction [25].

Many authors have noticed the association of hypokalemia with severe outcomes in COVID-19, as shown by the results of our study. Hypokalemia is a common electrolyte disorder in this viral infection and most likely occurs as multifactorial. Respiratory alkalosis caused by hyperventilation, as well as the use of diuretics, gastrointestinal loss, etc., is stated as a possible cause [26]. Chinese authors also mentioned the use of corticosteroid therapy as a possible cause of hypokalemia while, in addition, Italian authors emphasized the loss of K through urine measured during 24 hrs [26, 27]. Further additional research will certainly contribute to clarifying the occurrence of this electrolyte disorder.

If we look at the variables that were isolated in patients who went from mild to severe clinical picture due to the deterioration of the general condition, we can see that the variables showing significance largely coincided with the variables that stood out as prognostic factors during the examination of the severe clinical picture and fatal outcomes.

Conclusion

Our research showed that some routine clinical laboratory parameters, such as BMI, PT, albumin levels, CRP, and LDH, may have an important role in the early detection of patients developing severe clinical pictures and fatal outcomes. Lung X-ray showed predictive significance in all clinical forms with more severe outcomes. Similarly, the same parameters besides ferritin may promptly indicate the progression of clinical symptoms and signs in patients with an initially mild clinical picture. That is why monitoring these parameters is essential for the timely clinical assessment of patients with COVID-19 and, thus, the timely application of adequate therapeutic protocols in the treatment of these patients.

Conflict of interest

No potential conflict of interest was reported by the authors.

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Quality of life assessment in patients treated due to chronic pancreatitis

Procena kvaliteta života bolesnika lečenih zbog hroničnog pankreatitisa

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Abstract

Background/Aim. Chronic pancreatitis (CP) causes inflammatory changes in the tissue of the pancreas, resulting in irreversible tissue damage. Pain, endocrine, and exocrine pancreatic insufficiency develop, thereby reducing the quality of life (QoL) of patients. The aim of the study was to determine the significance of surgical treatment in improving the QoL of patients with CP. **Methods.** QoL assessment of 50 patients diagnosed with CP was performed using a certified Euro Quality of Life-5 Dimension-5 Level (EuroQol-5D-5L) Questionnaire translated into Serbian. According to the method of treatment, patients completed the questionnaire. Patients were divided into two groups (conservatively treated – CT and surgically treated – ST), and all comparisons were made between groups. **Results.** Patients in stage B of CP were divided into two groups of 25 patients. The mean age in the ST group was 48.56 ± 11.91, and in the CT group was 51.08 ± 11.61 ($p = 0.452$). The male/female ratio in the ST group was 18/7, and in the CT group was 22/3 ($p = 0.289$). Pain in the ST group was present in 23 patients, and in the CT group was present in 18 patients ($p = 0.141$). Loss of appetite in the ST group was present in 7 patients and in the CT group in 10 patients ($p = 0.256$). Weight loss in both groups was equal ($p = 1.000$). Based on the EuroQol-5D-5L Questionnaire, significant differences were found ($p < 0.001$) between groups in Mobility and Pain/Discomfort, in Anxiety/Depression ($p = 0.003$), in Self-care ($p = 0.004$), and in Usual activities ($p = 0.008$). **Conclusion.** CP significantly reduces the QoL of patients treated either conservatively or by surgical approach. This study showed that surgical treatment is more beneficial for the QoL of patients with CP than the conservative approach.

Key words:
drug therapy; pancreatitis, chronic; quality of life; surgical procedures, operative; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Hronični pankreatitis (HP) izaziva zapaljenske promene u tkivu pankreasa, što dovodi do nepovratnog oštećenja tkiva. Razvijaju se bol, endokrina i egzokrina insuficijencija pankreasa, čime se smanjuje kvalitet života (KŽ) bolesnika. Cilj rada bio je da se utvrdi značaj hirurškog lečenja za poboljšanje KŽ bolesnika sa HP. **Metode.** Procena KŽ 50 bolesnika kojima je dijagnostikovan HP izvršena je korišćenjem sertifikovanog upitnika *Euro Quality of Life-5 Dimension-5 Level* (*EuroQol-5D-5L*) prevedenog na srpski jezik. Bolesnici su popunili upitnik, a zatim podeljeni u dve grupe (konzervativno lečeni – KL i hirurški lečeni bolesnici – HL) između kojih su izvršena sva poređenja. **Rezultati.** Bolesnici u B stadijumu HP podeljeni su u dve grupe od po 25 bolesnika. Prosečna starost u HL grupi iznosila je 48,56 ± 11,91 godina, a u KL grupi 51,08 ± 11,61 godina (p = 0,452). Odnos muškaraca i žena u HL grupi bio je 18/7, a u KL grupi 22/3 (p = 0,289). Bol u HL grupi bio je prisutan kod 23 bolesnika, a u KL grupi kod 18 bolesnika (p = 0,141). Gubitak apetita u HL grupi bio je prisutan kod 7 bolesnika, a u KL grupi kod 10 bolesnika (p = 0,256). Smanjenje telesne mase bilo je jednako u obe grupe (p =

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1,000). Na osnovu EuroQol-5D-5L upitnika utvrđene su značajne razlike (p <0,001) između grupa u pokretljivosti i bolu/nelagodnosti, anksioznosti/depresiji (p = 0,003), u samopomoći (p = 0.004), i u uobičajenoj aktivnosti (p = 0,008). **Zaključak.** HP značajno smanjuju KŽ bolesnika koji se leče i konzervativnim i hirurškim pristupom. Ova studija je pokazala da je hirurško lečenje korisnije za KŽ od konzervativnog lečenja kod bolesnika sa HP.

Ključne reči:
lečenje lekovima; pankreatitis, hronični; kvalitet života; hirurgija, operativne procedure; ankete i upitnici.

Introduction

Chronic pancreatitis (CP) is an inflammatory disease characterized by irreversible damage to the pancreatic tissue caused by chronic inflammation, loss of acinar cells, and sclerosis. The functional impairment comprises the reduced secretion of digestive enzymes and the gradual exocrine and endocrine pancreatic insufficiency causing pain, maldigestion, and loss of body mass, consequently leading to the deterioration of the quality of life (QoL) [1–7]. CP development is related to alcohol abuse, smoking, and poor living conditions [1–4], and apart from the individual, CP bears significant social and economic impact due to its prevalence of 27.4 cases per 100,000 people [1–3].

As CP is an incurable, progressive disease, the treatment is aimed at improving symptoms and QoL. Initial treatment for uncomplicated cases is usually conservative and includes alcohol and smoking cessation, the use of supplements including pancreatic enzymes, proton pump inhibitors, and symptomatic treatment. Surgical and interventional procedures are reserved for the cases with CP resistant to conservative treatment and complications, which are quite common (increased intrapancreatic pressure, pseudocysts, fistulas or abscesses, stenosis of the duodenum and bile duct, calculus, vascular disorders, and neoplasms) [5–10].

One of the most important indicators of successful treatment of CP is QoL [11, 12]. The CP reduces QoL, with a significant impact on mental and physical health [12–14]. Various instruments were employed for the assessment of QoL in these patients with variable relevance. Several studies confirmed that pain is the most important single contributor to QoL decrease. With higher stages of CP, the pain is more intense, and, therefore, the QoL is worse [11–13, 15]. In addition to chronic pain, malnutrition poses an additional significant factor. Based on published data, the surgical or endoscopic approach in treating CP patients efficiently decreases symptoms including pain, nausea, and fatigue and consequentially improves the QoL [13, 15–23]. Nevertheless, no ideal procedure or specific guidelines for the treatment of CP are available, and the decision on using specific surgical procedures (drainage, resection, drainage and resection, denervation, etc.) is complicated [17–24].

The aim of this study was to evaluate QoL in surgically and conservatively treated patients with CP using the Euro Quality of Life-5 Dimension-5 Level (EQ-5D-5L) Questionnaire, as well as to prove that the EQ-5D-5L is easy to perform, that it is not a long-term questionnaire and may be useful in assessing the transition from conservative to surgical treatment of patients with CP in clinical practice.

Methods

Study design

The study protocol was approved by the Ethics Committee of the Military Medical Academy in Belgrade (November 2, 2018), and informed patient consent was obtained. The study was conducted as a cross-sectional observational study and included 50 patients with CP treated for over 10 years, from January 1, 2008, to December 31, 2017. Two 25-patient groups were defined based on the treatment applied – patients treated with surgical procedures and patients treated with conservative treatment at the Clinic for General Surgery and Clinic for Gastroenterology of the Military Medical Academy in Belgrade.

The study inclusion criteria were the diagnosis of CP based on symptom persistence, biochemical and radiological analyses in stage B, patients aged between 18 and 80 years, in a good living environment, with a history of discomfort of at least two years, and patients expected to respond to therapy without paradoxical events. Exclusion criteria from the study were the inability to give informed patient consent to participate in the study, patients who did not wish to participate in the survey, and mentally disabled patients. Patients completed the questionnaire independently. An investigator ensured that all questions were answered; all data were kept confidential.

Quality of life assessment

For the QoL assessment, we used the validated EQ-5D-5L Questionnaire, which was translated into Serbian and approved by the EuroQoL Group [25]. The EQ-5D-5L Questionnaire contains five dimensions: mobility, personal care, usual activities, pain/discomfort, and anxiety/depression. In each dimension, patients are classified into five functional levels [25]. The respondent marks one of the cubes which most closely describes his or her health status on the day of the interview in each of the five dimensions. Each answer is evaluated by a single digit from 1 to 5 [25].

The second instrument in the assessment was a Visual Analogue Scale (VAS), 20 cm long, marked from 0 to 100. The worst health imaginable is marked with 0 and the best with 100 [25]. Following the patient's informed consent about data confidentiality and participation in the study, they were familiarized with the survey and its filling. Patients completed the questionnaire independently without the presence of medical staff.

Statistical analysis

All attribute variables were presented in the form of frequencies of particular categories, and statistical significance between the categories was tested by the χ^2 test. All continuous variables are presented as mean ± standard deviation (SD). The Mann-Whitney *U* test or Student *t*-test were applied for differences in continuous variables, depending on the normality of the distribution based on the Kolmogorov-Smirnov test. All analyses were estimated at the level of statistical significance of $p < 0.05$. The complete statistical analysis of the data was done in the statistical computer program PASW Statistics, version 18 (IBM Corporation, Armonk, New York).

Results

The study included data provided from 50 patients with CP treated in our hospital. Patients were divided into two groups: the conservatively treated group – CT (25 patients) and the surgically treated group – ST (25 patients). The demographic and clinical characteristics of patients within the two groups are shown in Table 1.

There was no significant difference between groups regarding the demographic characteristics and risk factors, as well as regarding the clinical symptoms (pain, loss of appetite, appearance of stool, and weight loss). Pain was the dominant symptom in 41 (82%) patients of both groups.

The complete data on surgical procedures are presented in Table 2.

All patients completed a 30-min EQ-5D-5L Questionnaire. There was a statistically significant difference between patient groups in all dimensions of the EQ-5D-5L Questionnaire (Table 3).

The EQ-5D-5L Questionnaire showed better results in the ST group. There were no patients with level 5 disturbances of the QoL. Severe problems were present in 9 patients in the CT group, while in the ST group, there were no patients who reported moderate problems (except the one with moderate Depression/Anxiety).

A significant difference was found between groups in all five dimensions (Mobility, Self-care, Usual activities,

Table 1

Demographic characteristics, risk factors, and clinical symptoms in patients with chronic pancreatitis

Parameters	Surgically treated	Conservatively treated	*p*-values
Age (years), mean ± SD	48.56 ± 11.91	51.08 ± 11.61	0.452#
Sex (male/female), n	18/7	22/3	0.289*
Alcohol consumption, n	13	17	0.386#
Smoking, n	17	19	0.753#
Pain, n	23	18	0.141*
Loss of appetite, n	9	14	0.256*
Appearance of stool changed, n	7	10	0.593#
Weight loss, n	12	12	1.000*

SD – standard deviation; n – number of patients.
***χ^2 test; #Mann-Whitney *U* test.**

Table 2

Surgical procedures performed

Parameter	Patients (n = 25) n (%)
Type of surgery	
drainage	11 (44)
resection	5 (20)
combined	8 (32)
neurolysis	1 (4)
Procedure	
celiacolysis	2 (8)
distal resection and splenectomy	3 (12)
Fray and Berger	8 (32)
Partington-Rochelle	3 (12)
Roux	8 (32)
PPPD (pylorus-preserving pancreaticoduodenectomy)	1 (4)

n (%) – number (percentage) of patients.

Table 3

EQ-5D-5L distribution by groups

Parameter	Mobility, n (%)	Self-care, n (%)	Usual activities, n (%)	Pain/Discomfort, n (%)	Anxiety/Depression, n (%)
Level 1					
CT	8 (32)	16 (64)	15 (60)	1 (4)	4 (16)
ST	24 (96)	25 (100)	24 (96)	12 (48)	14 (56)
Level 2					
CT	13 (52)	7 (28)	7 (28)	13 (52)	10 (40)
ST	1 (4)	-	1 (4)	13 (52)	10 (40)
Level 3					
CT	2 (8)	2 (8)	-	9 (36)	9 (36)
ST	-	-	-	-	1 (4)
Level 4					
CT	2 (8)	-	3 (12)	2 (8)	2 (8)
ST	-	-	-	-	
Level 5					
CT	-	-	-	-	-
ST		-	-	-	-
p-value*	< 0.001	0.004	0.008	< 0.001	0.003*

CT – conservative treatment; ST – surgical treatment; EQ-5D-5L – Euro Quality of Life 5 Dimension-5 Level Questionnaire; Level 1 – no problems; Level 2 – mild problems; Level 3 – moderate problems; Level 4 – severe problems; Level 5 – extreme problems.
***χ^2-test.**

Pain/Discomfort, and Anxiety/Depression). Based on the EQ-5D-5L Questionnaire, pain and mobility were significantly better in the ST group than in the CT group ($p < 0.001$). The most notable difference between groups was noted in pain ($p < 0.001$). Surgically treated patients seldom had problems regarding mobility, self-care, and performing usual daily activities.

Pain measured by VAS showed a statistically significant difference ($p < 0.001$) in relation to good or poor health at the time of completing the questionnaire (Figure 1).

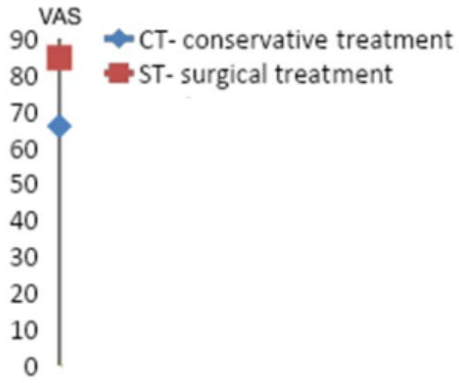

Fig. 1 – Visual Analogue Scale (VAS) on the day of completing the questionnaire marked from 0 to 100 (Independent samples *t*-test).

Discussion

This is the first study to evaluate QoL in patients with CP using a short form general questionnaire – EQ-5D-5L, an easy to perform and not a time-consuming questionnaire, providing an adequate sense of the actual QoL of these (sometimes demanding) patients.

Statistically significant differences were found between surgically and conservatively treated patients in all five dimensions of the Questionnaire, regardless of the type of surgical intervention.

Instruments for measuring the QoL and pain intensity include the European Organization for Research and Treatment of Cancer Quality Life Questionnaire Core 30 (EORTC QLQ-C30), Gastrointestinal Symptom Rating Scale, Short-Form McGill Pain Questionnaire (SF MPQ), QLQ-PAN26, PROMIS inventories for pain interference, depression, and anxiety, Visual analog scale (VAS), and Izbicki score. These belong to general and pancreatitis-specific inventories, and some are demanding to complete [26, 27]. Recently, major studies implemented SF12, which was later revised to version 2, and SF8 was also developed [27–35]. Patients with CP are usually not in the mood to complete questionnaires, especially when without symptoms; therefore, we aimed to evaluate our outcomes using EQ-5D-5L Questionnaire as the simplest but sufficient tool for the QoL assessment [25]. This allowed the evaluation of even the most demanding patients.

The results of North American Pancreatitis Study 2 (NAPS-2) show that CP reduces QoL, with a significant impact on mental and physical health. Pain intensity and metabolic changes in patients with CP are determining factors in the QoL [12, 15, 26]. Our study confirmed the significance of pain, as well as anxiety, and/or depression for the QoL. In the majority of the patients, these symptoms resolved after the surgery, while in the CT group, the majority of the patients remained with at least slight disturbance in all but the self-care dimension.

Just over half (52%) of CT patients reported slight mobility problems, while there were 8 patients (32%) with no problems and 8% of patients who reported moderate and severe disturbances each. This is probably in relation to the pain presence. No previous studies on CP patients reported results on mobility impairment as a separate aspect of QoL; it is usually assessed within the home/work performance.

The comparison of these in the most commonly used SF36 questionnaire appears inappropriate, as this performance was more pronounced due to the psychological impairment rather than the physical. Another component that might be considered is energy, which was not found to be significantly improved with surgery in other studies [33].

The majority of both ST and CT patients preserved their self-care abilities with no problems (41 out of 50). There were, however, 9 patients –7 with slight and 2 with moderate disturbances, all within the CT group. Although this difference was not statistically significant in our 50-patient study, previous studies reported significant improvements within this component [29–33].

The results regarding the usual activities are similar – 3 patients remained with severe limitations in the performance of the usual activities within the CT group. The results are also in order with the previous studies reporting the significant improvement in usual activities performance [30–33].

Pain and/or discomfort remained present in a slight form in a little over half (52%) of the operated patients, while all but one patient in the CT group remained in pain (2 severe). The remaining slight pain had no impact on the functional performance of ST patients. Although previous studies showed significant improvement in pain as the most notable effect of the surgery, the majority of surgical procedures are aimed at pain treatment [28–33, 35]. Some studies separated the pain entity into general and pancreatic due to the specificity of the questionnaire [29, 32]. This pancreatic pain was found to influence all other components of the SF36 inventory [34].

Although the main focus of the QoL assessment in CP remains on the physical health and physical functional outcome, mental status is also a significant (but smaller) factor in the overall QoL. In the ST group, we achieved complete symptom resolution in 56% of the patients, while the slight and moderate disturbances were present in 40% and 4% of the patients, respectively. Previous studies have found that the mental component is also significantly influenced by the comorbidity status; moreover, it remained significant even after correction for confounding factors [12, 34].

When considering patients who underwent Fray's procedure in CP treatment, there was pain relief and thus an improvement in QoL in 70% to 80% of cases [28]. Compared to other traditional surgical techniques, the Frey procedure with additional pancreatic head resection offers advantages regarding a long-term pain-free state and low risk for developing complications after the surgical treatment. Therefore, this procedure can be recommended as a standard method of surgical treatment of CP [30]. Additionally, there are several modifications of the Fray procedures, such as the Berne, Izbicki, and Imaizumi, which have been used successfully to improve the QoL of CP patients [28, 31, 35]. A study of 100 patients with CP, surgically treated using the Bern procedure, showed a low postoperative mortality rate (1%) and a low postoperative complications rate (16%) [23]. In a sample of patients, 55% had lower pain, and 67% had increased body weight postoperatively. The results of CP treatment using the Berne procedure were thus deemed excellent [35]. The QoL of patients after the surgical procedure is better than the QoL of patients with CP who receive only the conservative treatment in our study regardless of the procedure; however, the majority of our patients received the Frey procedure.

The limitations of this study include the relatively small group of patients, the fact it was a single-center raising concerns for bias, and that no standardized surgical procedure was performed, probably compromising the surgical treatment results.

Further studies, preferably randomized controlled trials in larger groups of patients, are needed to confirm the value of the EQ-5D-5L Questionnaire in assessing the QoL in patients with CP and to compare the results with other inventories. Furthermore, a standardized surgical approach is mandatory before conducting the trial.

Conclusion

The EQ-5D-5L Questionnaire appears easy to perform, not time-consuming, and provides insight into the patient's real-life performance, therefore being accessible to the most inapproachable patients.

Surgical treatment in patients with CP significantly improves patient's functional outcome in terms of mobility, self-care, and usual activities, compared with CT patients. Although there is no consensus nor relevant guidelines for the adequate assessment of QoL in patients with CP, the EQ-5D-5L Questionnaire may be a useful tool for the initial evaluation of these patients and subsequent assessment of treatment performance.

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Association between cancer surface area and histopathological parameters of laryngeal squamous cell carcinoma in total laryngectomy specimens

Veza između površine tumora i histopatoloških parametara karcinoma skvamoznih ćelija larinksa u uzorcima totalne laringektomije

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Abstract

Background/Aim. Numerous histopathological parameters, such as cartilage penetration, perineural and lymphovascular invasion, presence of metastatic tissue in regional lymph nodes (LNs), extranodal extension (ENE) of nodal metastases, as well as the presence of cancer tissue on resection borders, are all important factors influencing survival in patients with laryngeal squamous cell carcinoma (LSCC). The aim of the study was to determine if there is an association between cancer surface area (CSA) and these histopathological characteristics. The presence of ENE of metastatic tissue in regional LNs was also investigated. **Methods.** In a retrospective study, one hundred and forty cases of LSCC were revised and processed after total laryngectomy. The cases were found in the archives of the Histopathology Laboratory of the Clinic for Otorhinolaryngology and Maxillofacial Surgery, University Clinical Center of Serbia. **Results.** A significant difference was found in CSA depending on cancer penetration into the thyroid cartilage, perineural invasion, and positive resection margins. Cancers with larger CSA were more common in the advanced T stage. Metastases were found in 36 out of 72 (50%) neck LN samples submitted for evaluation. The difference in CSA was also found depending on the presence of metastatic tissue in regional LNs. ENE was present in 69.4% of involved LNs, and it was more frequent in LNs 3 cm in size or larger. **Conclusion.** There is a significant difference in CSA depending on the presence of cartilage penetration, perineural invasion, presence of cancer tissue on resection borders, and presence of metastases in regional LNs. Larger cancers tend to be of a higher T stage. ENE is more common in LNs 3 cm in size or larger.

Key words:
carcinoma, squamous cell; laryngectomy; laryngeal neoplasms; lymph nodes; lymphatic metastasis; neoplasm staging; prognosis; tumor burden.

Apstrakt

Uvod/Cilj. Mnogobrojni histopatološki parametri skvamocelularnog karcinoma larinksa (LSCC), kao što su proboj malignog tkiva u hrskavicu larinksa, perineuralno i limfovaskularno širenje, prisustvo metastaza u regionalnim limfnim čvorovima (LN), pojava ekstranodalne ekstenzije (ENE) metastatskog tkiva, kao i prisustvo malignog tkiva na linijama resekcije nakon hirurškog odstranjivanja tumora, su važni faktori koji utiču na preživljavanje bolesnika sa LSCC. Cilj rada je bio da se utvrdi da li postoji povezanost između površine tumora (*cancer surface area,* CSA*)* i pomenutih histopatoloških karakteristika. Ispitana je i učestalost pojave ENE u regionalnim LN vrata zahvaćenih metastazom. **Metode.** U retrospektivnoj studiji analizirano je 140 slučajeva LSCC obrađenih nakon totalne laringektomije, pronađenih u arhivi Patohistološke laboratorije Klinike za otorinolaringologiju i maksilofacijalnu hirurgiju Univerzitetskog kliničkog centra Srbije. **Rezultati.** Utvrđena je značajna razlika u CSA u zavisnosti od toga da li je postojao proboj tumora u tireoidnu hrskavicu larinksa, perineuralno širenje i prisustvo malignog tkiva na linijama resekcije. Karcinomi sa većom CSA bili su i višeg T stadijuma. Metastaze su bile prisutne u 36 od ukupno 72 (50%) dostavljena uzorka LN vrata. Postojala je razlika u CSA u zavisnosti od prisustva metastaza u regionalnim LN. Kod 69,4% zahvaćenih LN bila je prisutna ENE i to značajno češće u LN veličine 3 ili više cm. **Zaključak.** Postoji statistički značajna razlika u CSA u zavisnosti od prisustva

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proboja u tireoidnu hrskavicu larinksa, perineuralnog širenja, prisustva malignog tkiva na linijama resekcije i prisustva metastaza u regionalnim LN. Veći tumori imaju višu vrednost T stadijuma. Ukoliko su zahvaćeni LN bili prečnika 3 cm ili veći, ENE je bila češća.

Ključne reči:
karcinom, planocelularni; laringektomija; larinks, neoplazme; limfne žlezde; neoplazme, limfatska metastaza; neoplazme, određivanje stadijuma; prognoza; tumorsko opterećenje.

Introduction

Laryngeal cancer (LC) is, after lung cancer, the second most common cancer of the respiratory tract, with around 40 thousand new cases in Europe each year [1]. It most frequently affects men in their sixth and seventh decade of life [2]. The most common histological type (over 95%) is squamous cell carcinoma (SCC) [3]. LC stage is determined using the tumor, node, metastasis (TNM) cancer staging system, with certain differences between clinical and pathological assessment of the N stage.

Therapy of LC depends on clinical presentation and cancer stage. Total laryngectomy followed by radiotherapy is still the leading treatment option for advanced LCs (of T3 and T4 stage), though advantages of combined chemoradiation have been shown in certain cases [3–6].

Prognostic factors linked to shorter survival after surgery and radiotherapy are the supraglottic primary site, presence of metastases in neck lymph nodes (LNs) and extranodal extension, as well as high mitotic index and aggressive histological pattern of invasion [7–10]. Cancers smaller than 2.5 cm and cancers completely surgically removed carried a significantly better prognosis [9, 11].

One of the most important independent prognostic factors for the survival rate of patients with LC is the spread of metastatic tissue beyond the limits of the affected LN (ENE). The risk of ENE is related to the higher N stage of cancer and the diameter of the largest metastatic LN [12, 13].

All of the data significant for diagnosis and prognosis is obtained only after surgical resection and histopathological evaluation of laryngeal and neck tissue. However, the size of the cancer is easily obtainable information, which may be useful in assessing the prognosis of these patients.

The aim of this study was to determine whether there is a statistically significant connection between cancer surface area (CSA) and other histopathological parameters, which are important for diagnosis, treatment, and prognosis in patients with LC. The presence of ENE in metastatic LNs was also determined.

Methods

One hundred and forty total laryngectomy samples were selected from archives of the Histopathology Laboratory of the Clinic for Otorhinolaryngology and Maxillofacial Surgery, University Clinical Center of Serbia. The samples were obtained from January 1, 2016, to November 1, 2018.

The data necessary for this study were collected from the micro- and macroscopic descriptions, which are part of standard histopathological reports. Cases were reviewed with regard to general patients' data, dimensions, cancer surface area, differentiation and type of cancer, presence of perineural and lymphovascular invasion, penetration into the thyroid cartilage, presence of cancer tissue on resection margins, and presence of metastases in regional LNs. In cases where metastatic tissue was found in the LNs, data on the diameter of the largest involved LN and ENE was collected.

CSA is a parameter of the cancer size and was calculated by multiplication of maximum length and maximum width of tumor in millimeters, estimated from the cross sections of cancer tissue samples made by a pathologist (Figures 1a and 1b).

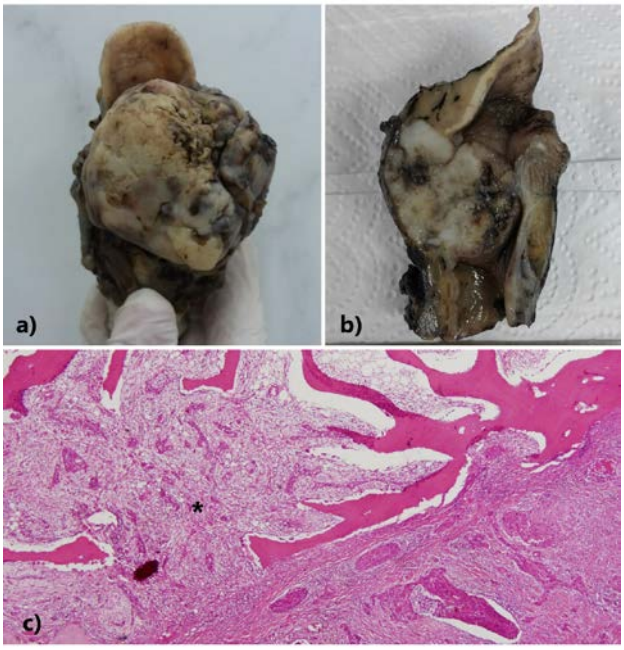

Fig. 1 − a) and b) Gross image of transglottic bilateral laryngeal squamous cell carcinoma; c) Ossified thyroid cartilage infiltrated by squamous cell carcinoma (*hematoxylin and eosin staining, original magnification ×400).

Data were processed by descriptive and analytical statistic methods in the IBM SPSS Statistics program (V21.0.0). Data with normal distribution were described by the arithmetic mean and standard deviation, while data with non-normal distribution were described using median, minimum, and maximum values. Categorical variables were described using frequencies. Based on the type of data, the following tests were used: Spearman rank order correlation, Mann-Whitney *U* test, Kruskal-Wallis test, χ^2 test, and Student's *t*-test.

Results

Out of the 140 patients with laryngeal squamous cell carcinoma (LSSC), 120 were male (85.7%) and only 20 were female (14.3%). Patients' ages ranged from 38 to 83 years, with a mean age of 65.41 ± 7.16 years. The average CSA was 860 mm^2 (225−4,680 mm^2).

In 81 cases, cancer was staged as T4 (57.9%), in 57 cases, it was T3 (40.7%), and only two cases were of T2 stage (1.4%). The average CSA of T4 stage cancers was 1,222 mm^2, of T3 stage cancers 912 mm^2, and of T2 stage cancers 291 mm^2. The smallest cancer to penetrate laryngeal cartilage had a CSA of 300 mm^2. There was a significant difference in CSA based on cancer T stage; namely, cancers of higher T stage were larger (Figure 2, $p = 0.005$).

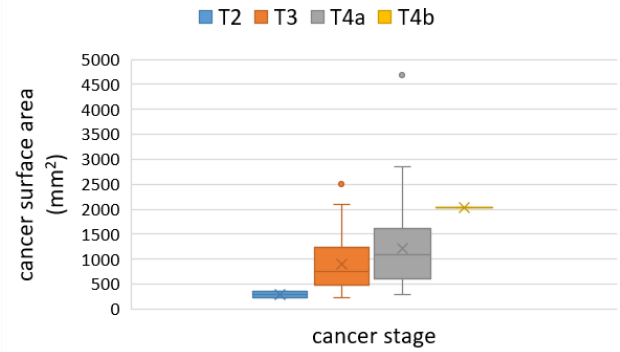

Fig. 2 – Cancers of higher tumor (T) stage had larger cancer surface area (as per Kruskal-Wallis test, $p = 0.005$).

No correlation was found between CSA and the age of the patients ($p = 0.863$). No statistically significant difference in CSA was found depending on cancer differentiation ($p = 0.153$) and histological type ($p = 0.963$). On the contrary, a significant difference in CSA was found depending on the presence of penetration of tumor tissue into the laryngeal cartilage (Figure 1c), i.e., cancers that penetrated the cartilage more often had a larger CSA value (Figure 3, $p = 0.001$). Cancers with larger CSA were also linked to a more frequent perineural invasion (Figure 4, $p = 0.005$). In all the total laryngectomy samples, a lymphovascular invasion was present. A significant difference in CSA was also found depending on the presence of cancer tissue on the resection margins, or less than 0.5 mm distance from them (Figure 5, $p = 0.031$).

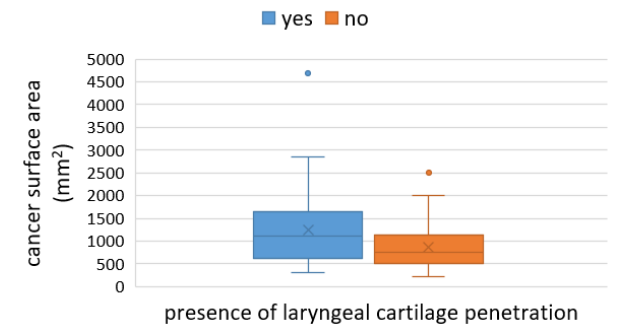

Fig. 3 – Laryngeal cartilage penetration was more often in cancers of larger surface area (as per Mann-Whitney *U* test, $p = 0.001$).

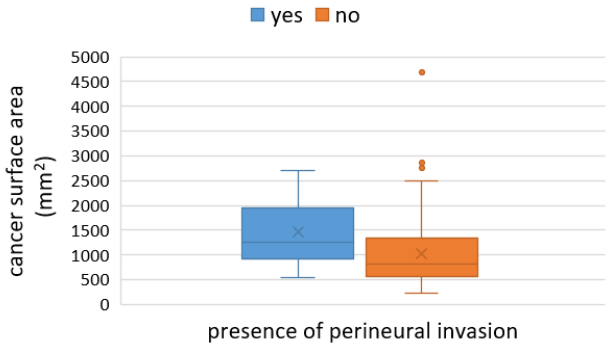

Fig. 4 – The presence of perineural invasion was more often in cancers of larger surface area (as per Mann-Whitney *U* test, $p = 0.005$).

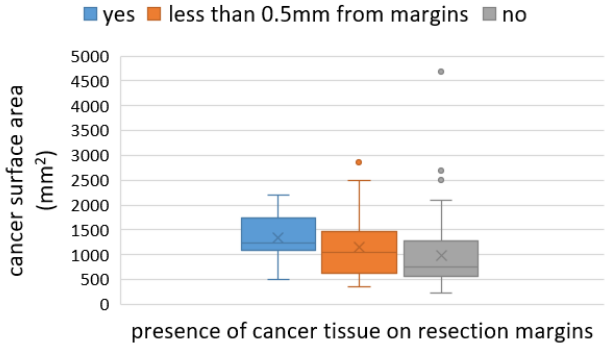

Fig. 5 – Incidence of the presence of cancer tissue on resection margins grew with cancer surface area (as per Kruskal-Wallis test, $p = 0.031$).

Out of 140 cases, LNs were submitted to analysis with 72 total laryngectomy samples (51.4%). LC metastases were found in 36 out of 72 LNs (50%). A significant difference in CSA was found depending on the presence of metastases in LNs (Figure 6, $p = 0.001$).

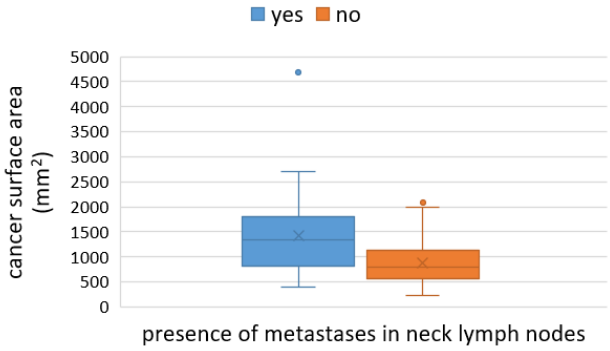

Fig. 6 – Metastases in neck lymph nodes were more often found in cancers of larger surface area (as per Mann-Whitney *U* test, $p = 0.001$).

Out of 36 cases with LN involvement, ENE was found in 25 cases (69.4%). No significant association was found between CSA and the presence of ENE. However, it was determined that ENE was more likely to occur if the largest diameter of the affected LN is 3 cm or more than if it is less than 3 cm (Table 1, $p = 0.002$).

Table 1

Extranodal extension (ENE) was more frequent in lymph nodes (LNs) 3 cm in size or larger (as per χ^2 test, $p = 0.002$)

ENE	LN diameter < 3 cm	LN diameter ≥ 3 cm
+	4	20
-	9	3

+ – ENE present; - – ENE absent.

Discussion

This study shows that CSA is significantly related to several prognostic factors: cancer stage, cartilage penetration, presence of perineural invasion, presence of cancer tissue on resection margins, and presence of metastases in LNs.

Association between CSA and cancer stage is of great importance since the cancer T stage is directly linked to patients' survival rates. In a study published in Canada in 2012, the two-year survival rate for T3 stage cancer was 89% and 60% for T4 stage [14]. Another study published in Germany in 2011 showed similar results (80% for the T3 stage and 64% for the T4 stage) [15].

Penetration of cancer tissue into the thyroid cartilage indicates a worse prognosis and is linked to more frequent cancer recurrence [16, 17]. The significance of cartilage penetration is also seen in the fact that its presence is one of the criteria for the classification of cancer in stage T4 [7].

Presence of perineural invasion is another prognostic factor associated with shorter survival and increased incidence of local recurrence [18, 19].

Cancer tissue was present on the resection margins in 10 cases (7.1%), and in 37 (26.4%), it was very close to resection margins, i.e., less than 0.5 mm from them. The presence of cancer tissue on resection margins is associated with a more frequent recurrence of cancer, as well as shorter survival [20, 21].

As already noted, metastases in neck LNs are more often if cancer has a higher CSA value, as was shown in other studies. It is also suggested that T3 and T4 stage cancers give regional metastases in more than 50% of the cases [22], which agrees with our results. Metastases were present in exactly 50% of the cases in which LNs were delivered for examination.

CSA is linked to shorter survival as an independent prognostic factor as well. A study conducted in Spain showed that cancers with the largest diameter of 2.5 cm or less carry a significantly better prognosis [9].

ENE was present in 25 of 36 cases with affected neck LNs (69.4%). Different studies have linked the presence of ENE with the size of the metastatic LN; more precisely, ENE occurs more frequently if the diameter of the LN is 3 cm or larger. Results obtained in our study agree with this hypothesis. ENE is a significant prognostic factor associated with the presence of distant metastases and recurrence of cancer; both have an impact on shorter survival rates [12, 13].

Given that the size of LC has been associated with these histopathological parameters significant for the prognosis, clinical estimation of cancer size could itself have a certain prognostic value. The size of the cancer is, clinically, most accurately determined by different visualization methods, such as computerized tomography and nuclear magnetic resonance [23, 24].

The main limitation of this study was the lack of data on patients' survival that could be related to CSA. This way, we determined the significance of CSA on the prognosis only indirectly by studying the association between CSA and already known prognostic factors.

Conclusion

There is a significant difference in CSA depending on the presence of cartilage penetration, perineural invasion, the presence of cancer tissue on resection borders, and the presence of metastases in regional LNs. Larger cancers tend to be of a higher T stage. ENE is more common in LNs 3 cm in size or larger.

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GRACE, SYNTAX I, and SYNTAX II scores as predictors of one-year MACE in patients with myocardial infarction treated with percutaneous coronary intervention

GRACE, SYNTAX I i SYNTAX II skorovi kao prediktori jednogodišnjeg MACE kod bolesnika lečenih perkutanom koronarnom intervencijom

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Abstract

Background/Aim. The fundamental objective of primary percutaneous coronary intervention (PCI) in myocardial infarction is to provide early, complete, and sustained flow in the occluded artery that has led to myocardial ischemia or necrosis. The aim of this study was to determine the predictive power of a combination of GRACE, SYNTAX I, and SYNTAX II scores in predicting major adverse cardiovascular events (MACE) and one-year mortality in patients with ST-segment elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI) after primary PCI. **Methods.** The study included 400 patients who had their first acute myocardial infarction and underwent PCI. The patients were treated and followed for one year at the Clinical Hospital Center Zvezdara at the Department of Interventional Cardiology. By monitoring the defined clinical parameters, a comparative analysis of risk scores GRACE, SYNTAX I, and SYNTAX II was performed. Their sensitivity, specificity as well as predictive possibilities in predicting adverse outcomes were determined. **Results.** The incidence of MACE in our sample was 12.8%. Patients with STEMI entity had significantly higher values of GRACE, SYNTAX I, and SYNTAX II scores. The highest value for predicting the occurrence of MACE was shown by the SYNTAX II score (score value 29.3), with a sensitivity of 88.2% and a specificity of 76.8%. The GRACE score was a significant predictor of SYNTAX I and SYNTAX II scores. A two-way correlation was observed between the high score values of all three scores. **Conclusion.** The presented scores for the assessment of clinical and angiographic indicators showed good predictive power in assessing the outcome of adverse cardiovascular events in both clinical entities of acute myocardial infarction during one-year follow-up. By using the proposed scores to assess MACE, we can single out high-risk patients in order to prevent adverse events and reduce mortality. This suggests its suitability for clinical use in this patient population.

Key words:
myocardial infarction; percutaneous coronary intervention; prognosis; risk assessment; treatment outcome.

Apstrakt

Uvod/Cilj. Osnovni cilj primarne perkutane koronarne intervencije (PKI) kod infarkta miokarda je da obezbedi rani, potpuni i održivi protok u okludiranoj arteriji koja je dovela do ishemije ili nekroze miokarda. Cilj rada bio je da se utvrdi prediktivna snaga kombinacije GRACE, SYNTAX I i SYNTAX II skorova u predviđanju glavnih kardiovaskularnih neželjenih događaja (MACE) i jednogodišnjeg mortaliteta kod bolesnika sa ST-*segment elevation myocardial infarction* (STEMI) i *non*-STEMI (NSTEMI) nakon primarne PKI. **Metode.** Studijom je bilo obuhvaćeno 400 bolesnika koji su imali prvi akutni infarkt miokarda i bili podvrgnuti PKI. Bolesnici su lečeni i praćeni godinu dana u Kliničko-bolničkom centru Zvezdara na Odeljenju za interventnu kardiologiju. Praćenjem definisnih kliničkih parametara sprovedena je uporedna analiza skorova rizika: GRACE, SYNTAX I i SYNTAX II. Utvrđena je njihova senzitivnost, specifičnost kao i prediktivna vrednost u predviđanju

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neželjenih ishoda. **Rezultati.** Učestalost MACE u našem uzorku je bila 12,8%. Bolesnici sa STEMI entitetom imali su značajno veće vrednosti GRACE, SYNTAX I i SYNTAX II skorova. Najveću vrednost za predviđanje pojava MACE pokazao je SYNTAX II skor (vrednost skora 29,3) sa senzitivnošću 88,2% i specifičnošću 76,8%. Skor GRACE je bio značajan prediktor SYNTAX I i SYNTAX II skora. Zapažena je i dvosmerna korelacija između visokih vrednosti sva tri skora. **Zaključak.** Prikazani skorovi za procenu kliničkih i angiografskih pokazatelja pokazali su dobru prediktivnu moć u proceni pojave neželjenih kardiovaskularnih događaja kod oba klinička entiteta akutnog infarkta miokarda tokom jednogodišnjeg praćenja. Korišćenjem predloženih skorova za procenu MACE možemo izdvojiti visoko rizične bolesnike u cilju prevencije neželjenih događaja i smanjenja mortaliteta. To sugeriše njegovu pogodnost za kliničku upotrebu u ovoj populaciji bolesnika.

Ključne reči:
infarkt miokarda; perkutana koronarna intervencija; prognoza; rizik, procena; lečenje, ishod.

Introduction

The prevalence of cardiovascular disease (CVD) has been steadily increasing in recent decades, and the cause of half of all CVD deaths worldwide is an acute coronary syndrome (ACS). In Serbia, in 2017, CVD was the leading cause of death, with a share of 51.7% of total deaths, and 49.4% were deaths caused by ACS [1].

ACS is a condition of reduced coronary flow that leads to ischemia or necrosis of the corresponding part of the heart muscle [2]. This syndrome encompasses two entities: ACS with ST-segment elevation, which includes vasospastic angina and acute myocardial infarction – (STEMI), and without ST-segment elevation, which includes unstable angina pectoris and acute myocardial infarction (NSTEMI).

The prevalence of NSTEMI, according to research data, ranges from 4% to 13% [3], which is similar to the percentage of data in Serbia, where it is estimated that the number of new patients with acute myocardial infarction (AMI) is 179.8/100,000 [4]. Mortality from STEMI is associated with old age, Killip class, the time delay of treatment, limited capacity of emergency medical networks, care and treatment strategy, history of AMI, diabetes mellitus, renal failure, number of diseased coronary arteries, and left ventricular ejection fraction. Some studies have found that hospital mortality in people with STEMI is slightly higher than in people with NSTEMI (7% : 5%), but this difference decreases over six months by 12%: 13% [5]. The total mortality from AMI in Serbia is 12–13%, with a higher prevalence in patients with STEMI compared to NSTEMI, with a tendency to decrease mortality due to the use of percutaneous coronary intervention (PCI) and pharmacological therapy [4].

The basic mechanism of ischemia is significant occlusion of the coronary artery. Angiograms performed 4 hours after the onset of AMI indicate that STEMI most often has total coronary artery occlusion (84%), while in other cases, subtotal occlusion is present [6]. Pathological substrates differ in two forms of AMI: plaque rupture is present in 72% of STEMI and 32% of NSTEMI, while plaque erosion is present in 28% of STEMI and 48% of NSTEMI. Angiographic studies have shown that multivessel occlusion and culprit lesions in the circumflex artery are more common clinical findings in NSTEMI [7]. The primary goal of primary PCI in AMI is to provide early, complete, and sustained flow in the occluded artery that has led to myocardial ischemia or necrosis. A significant advantage of PCI is the reduction of the risk of intracranial hemorrhage, reduction of the occurrence of undesirable cardiovascular outcomes, improvement of left ventricular myocardial function, and improvement of the clinical outcome. Despite the reduction in mortality within 12 months after PCI by 4 –6% [8], patients remain at risk of adverse events after stent implantation [9].

Clinical experience indicates that in-hospital mortality increases if the "door to balloon" time is greater than 120 min and depends on the size and location of the infarction, age, previous coronary heart disease, risk factors, clinical characteristics, and comorbidities. Intrahospital mortality of patients with AMI-treated PCI is 0% to 13%, while mortality during the first year after PCI is higher (5%) than mortality in subsequent years (1%) [10].

In clinical practice, in order to determine the optimal time for the application of invasive AMI therapy, it is necessary to assess the degree of ischemic risk and determine the predictors of adverse outcomes. Complications after PCI include death, reinfarction, cerebrovascular stroke, emergency coronary artery bypass graft surgery, and various vascular complications (pseudoaneurysm, arteriovenous fistula, retroperitoneal bleeding, etc.) [11].

Numerous scoring systems have been developed with the aim of predicting the short-term and long-term risk of major cardiovascular adverse events (MACE) in patients with STEMI and NSTEMI [12]. In modern cardiology, a combination of scores is usually used to monitor as many significant clinical, angiographic, echocardiographic, and other parameters of clinical outcome in patients as possible in order to implement an optimal therapeutic strategy. The most commonly used predictive scores for short- and long-term risk of MACE in patients with AMI are the Global Registry of Acute Coronary Events (GRACE), the Synergy Between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery (SYNTAX) I, and SYNTAX II score [13, 14].

The aim of this study was to determine the reliability of clinical scores in assessing the risk of MACE in patients with STEMI and NSTEMI. The outcome designated as MACE in the paper refers to cardiac and cerebrovascular events such as mortality, recurrent myocardial infarction, revascularization procedure, and stroke.

Methods

The study was conducted as a prospective, observational study, and it enabled the monitoring of clinical parameters of GRACE, SYNTAX I and II scores in order to determine the short- and long-term risk of MACE in patients with STEMI and NSTEMI.

The study included 400 patients under the diagnosis of AMI, treated with PCI from January 1, 2018, to December 31, 2018, at the Clinical Hospital Center Zvezdara in Belgrade, at the Department of Interventional Cardiology. The diagnosis of AMI (STEMI and NSTEMI) was made based on the criteria for AMI defined by the consensus of the working group of the European Association of Cardiologists. Among patients, 68.5% had STEMI entity, while 31.5% of patients were diagnosed with NSTEMI entity.

Inclusion criteria for the patients in the study were the following: first myocardial infarction, STEMI and NSTEMI presentation, performed coronary arteriography within 12 hrs of diagnosis, and adequate patient compliance (data on regular use of dual antiplatelet therapy with acetylsalicylic acid and P2Y12 receptor inhibitors (clopidogrel/ticagrelor/prasugrel) within 12 months of revascularization (PCI).

Exclusion criteria for the patients from the study were the following: refusal of treatment and follow-up, age of patients < 18 and > 80 years, non-acceptance of the proposed revascularization procedure, the lethal outcome in the inpatient period, cardiogenic shock on admission, severe valvular heart defects, intracerebral tumor/aneurysm, active or recent internal bleeding, known hemorrhagic diathesis, contraindications to heparin and antiplatelet therapy, discontinuation of dual antiplatelet therapy before 12 months of PCI, other conditions leading to inflammatory conditions cardiac troponin values, terminal phase chronic renal failure, previous cerebrovascular stroke, previous myocardial infarction and revascularization procedures, and malignancies.

Instruments used in the research

The GRACE score was designed to stratify the risk in patients with ACS to predict hospital, six-month, and annual deaths [15]. It assesses eight independent variables: age, heart rate, systolic blood pressure, serum creatinine, Killip class on admission, the presence of ST abnormalities, cardiac biomarker values, and cardiac arrest on admission. The categorization of the GRACE score is represented by groups with low (≤ 108; < 1%), intermediate (109–140; 1–3%), and high risk (> 140; > 3%) for intrahospital, and low categories (≤ 88; < 3%), intermediate (89–118; 3–8%), and high risk (> 118; > 8%) for six-month mortality [16].

The SYNTAX I monitors the qualitative and quantitative parameters of coronary arteries in patients with AMI and is important in monitoring patients with stable multivessel coronary disease [17] but also in patients with STEMI, where the SYNTAX is a high score predictor of cardiovascular mortality after PCI [18]. Twelve angiogram parameters were monitored: dominance, number of lesions, localization, total occlusion, bifurcation, trifurcation, thrombosis, lesion length, tortuosity, severe calcification, diffuse disease, and aortic lesions. Scoring categorization is defined as low (≤ 22), intermediate (23–32), and high (> 33) risk.

The SYNTAX II score combines anatomical and clinical factors to predict post-procedural outcomes [19, 20]. It includes angiographic parameters as well as SYNTAX I with the addition of another parameter – "unprotected left main coronary artery" and clinical parameters: sex, age, creatinine clearance, left ventricular ejection fraction, peripheral vascular disease, and chronic obstructive pulmonary disease. The SYNTAX II score is categorized into three groups of mortality risk: low (≤ 22), moderate (23–32), and high (≥ 33) [21]. The SYNTAX risk score includes significant clinical parameters that are not present in the GRACE score and are significant independent predictors of mortality and MACE in patients with AMI.

The research was conducted in accordance with the Declaration of Helsinki. For the purposes of this study, the approval of the Ethics Commission of the Clinical Hospital Center Zvezdara in Belgrade was obtained on December 9th, 2019 (No 09-3174).

Data were analyzed in the SPSS Windows user package, version 19. The level of statistical significance was set to $p < 0.05$. The correlation between scores was examined by Spearman's correlation coefficient test. All variables that showed significant correlation with outcome variables in the univariate regression analysis were included in the multivariate regression analysis.

Linear and COX regression analyses were applied to identify factors associated with outcome variables in our study (SYNTAX I and SYNTAX II, MACE). The Kaplan-Meier test was used to calculate survival time after acute STEMI and NSTEMI entity. Finally, the Receiver Operating Characteristic (ROC) curve analysis was used to examine the validity of the GRACE score in the prediction of significant coronary occlusive disease, as well as the validity of the GRACE, SYNTAX I, and SYNTAX II scores in the prediction of MACE in individuals with STEMI and NSTEMI.

Results

The mean age of patients was 62.6 ± 10.8 years. Patients with NSTEMI were statistically significantly younger (p = 0.01) (60.7 ± 10.2 years) compared with patients with STEMI (63.5 ± 11.0 years). Positive family history was present in 69.0% of patients, and persons with STEMI entity had statistically significantly more associated chronic diseases (p = 0.02). The average body mass index in both groups of patients indicated the absence of obesity without a significant difference between these two groups of subjects (p = 0.8).

The mean value of the GRACE score in patients with NSTEMI entity was 107.9 ± 18.2, while the mean values of the SYNTAX I and SYNTAX II scores were 21.3 ± 4.9 and 22.8 ± 5.0, respectively. In patients with STEMI, the mean value of the GRACE score was 122.7 ± 20.8, while the

values of the SYNTAX I and SYNTAX II scores were 25.8 ± 4.9 and 27.5 ± 4.9, respectively. Statistical significance was observed (p = 0.001), and patients with STEMI entities had higher values of the GRACE, SYNTAX I, and SYNTAX II scores.

Examining the relationship between the GRACE and SYNTAX I and SYNTAX II scores, statistical significance was observed in the correlation of the value of the GRACE score with the SYNTAX I and SYNTAX II scores. In addition, the values of SYNTAX I and SYNTAX II scores in patients in the high-risk group for the development of adverse outcomes statistically significantly correlated (p < 0.01) with the values of the GRACE score.

According to the GRACE score, patients classified in the low-risk group belong to the same group according to the values of SYNTAX I (70.2%) and SYNTAX II (54.3%) scores, with the determining statistical significance. Statistical significance was also observed in the group of patients with medium risk for the development of adverse outcomes estimated according to the values of all three scores. When it comes to high-risk assessment, patients who were by the GRACE score classified into the high-risk group were statistically significantly correlated with the high-risk group according to values of the SYNTAX II score (62.3%), while according to the SYNTAX I score values were statistically significantly correlated with moderate risk group (65.2%) (Figure 1). A significant high degree of correlation was observed between the GRACE score and the SYNTAX I and the SYNTAX II scores (rho > 0.5; p = 0.001).

MACE and survival time of patients with STEMI and NSTEMI were monitored in patients treated with PCI. It was also observed that 51 patients (12.8%) had one of the MACE, namely: revascularization in 75.6% of patients, the fatal outcome in 15.7% of patients, while 3.9% experienced recurrent myocardial infarction and stroke. Among patients, 68.5% had STEMI entity, while 31.5% of patients were diagnosed with NSTEMI entity.

As shown in Table 1, patients with STEMI, the elderly, without a positive family history, with hypertension, Killip class II, cardiac arrest, higher heart rate, more

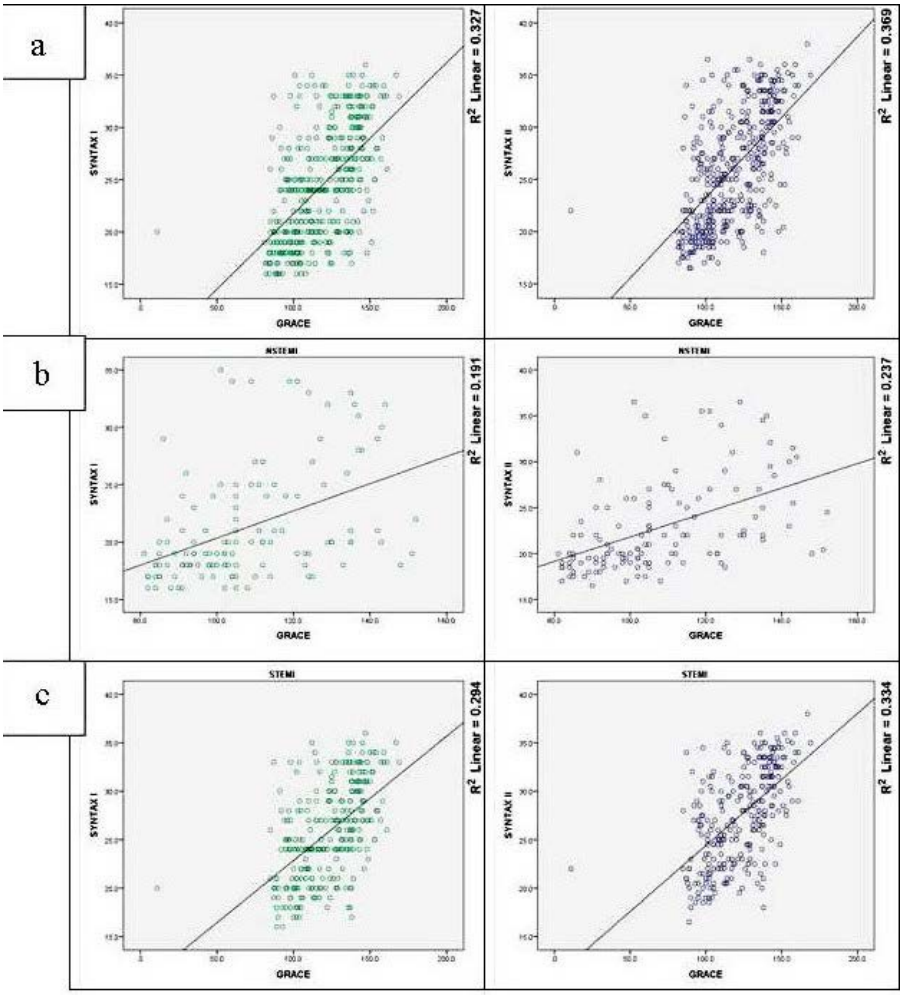

Fig. 1 – Correlation of GRACE score with SYNTAX I and SYNTAX II scores: a) whole sample of patients; b) in patients with NSTEMI entity; c) in patients with STEMI entity.

GRACE – Global Registry of Acute Coronary Events; SYNTAX – Synergy between PCI (percutaneous coronary intervention) with Taxus and Cardiac Surgery; NSTEMI – non-ST-elevation myocardial infarction; STEMI – ST-elevation myocardial infarction.

Table 1

Socio-demographic, clinical, and laboratory characteristics of patients with and without major adverse cardiovascular events (MACE) outcomes during the year

Variables	MACE		*p*
	no	yes	
Myocardial infarction entity, n (%)			
NSTEMI	117 (92.9)	9 (7.1)	0.02
STEMI	232 (84.7)	42 (15.3)	
Gender, n (%)			
male	232 (87.2)	34 (12.8)	0.97
female	117 (87.33)	17 (12.7)	
Smoking status, n (%)			
non-smoker	122 (84.7)	22 (15.3)	0.47
ex-smoker	115 (87.8)	16 (12.2)	
smoker	112 (89.6)	13 (10.4)	
Alcohol consumption, n (%)			
no	333 (87.2)	49 (12.8)	0.83
yes	16 (88.9)	2 (11.1)	
Positive family anamnesis, n (%)			
no	99 (81.8)	23 (18.9)	0.01
yes	250 (89.9)	28 (10.1)	
The presence of chronic diseases, n (%)			
no	44 (93.6)	3 (6.4)	0.007
diabetes	3 (100.0)	0 (0.0)	
hypertension	163 (92.6)	13 (7.4)	
GERBI	2 (100.0)	0 (0.0)	
hypothyroidism	2 (100.0)	0 (0.0)	
COPD	3 (100.0)	0 (0.0)	
more chronic diseases	132 (79.0)	35 (21.0)	
Angina pain or its equivalent, n (%)			
no	52 (85.2)	9 (14.8)	0.61
yes	297 (87.6)	42 (12.4)	
Killip class at the reception, n (%)			
one	331 (89.7)	38 (10.3)	0.001
two	18 (58.1)	13 (41.9)	
Cardiac arrest on admission, n (%)			
no	336 (88.4)	44 (11.6)	0.002
yes	13 (65.0)	7 (35.0)	
Isolated disease of the main tree, n (%)			
no	347 (87.4)	50 (12.6)	0.28
yes	2 (66.7)	1 (33.3)	
Number of diseased coronary arteries, n (%)			
one	172 (99.4)	1 (0.6)	0.001
two	108 (91.5)	10 (8.5)	
three and more	69 (63.3)	40 (36.7)	
Number of stents implanted, n (%)			
one	132 (90.4)	14 (9.6)	0.13
two	110 (88.7)	15 (11.3)	
three	58 (79.5)	15 (205)	
four and more	49 (86.0)	8 (14.0)	
Previous therapy, n (%)			
no	1 (0.3)	0 (0.0)	
antiaggregation	165 (85.5)	28 (14.5)	
antiaggregation + antihypertensive	44 (84.6)	8 (15.4)	
antiaggregation + hypolipidemic	106 (91.4)	10 (8.6)	
triple (antiaggregation, antihypertensive, hypolipidemic)	33 (86.8)	5 (13.2)	
Age (years), mean ± SD	62.17 ± 10.68	65.53 ± 11.26	0.03
Body mass index (kg/m^2), mean ± SD	23.28 ± 2.40	23.20 ± 2.72	0.81
Time from onset of pain to percutaneous coronary intervention (min), mean ± SD	127.53 ± 36.73	138.43 ± 38.44	0.05
Length of hospitalization (days), mean ± SD	7.55 ± 1.88	9.92 ± 3.59	0.001
Heart rate (beat/min), mean ± SD	81.03 ± 17.01	91.35 ± 22.67	0.003
Systolic admission pressure (mmHg), mean ± SD	130.79 ± 20.35	131.76 ± 20.73	0.75
Total cholesterol (mmol/L), mean ± SD	5.22 ± 1.32	5.24 ± 1.50	0.92
Triglycerides (mmol/L), mean ± SD	1.78 ± 1.00	1.95 ± 0.84	0.25
Tnl-ultra Troponin (ng/mL), mean ± SD	20.88 ± 15.38	20.64 ± 18.88	0.001
CRP (mg/L), mean ± SD	15.70 ± 19.15	23.21 ± 22.81	0.01
Glucose (mmol/L), mean ± SD	7.11 ± 2.76	8.57 ± 4.53	0.029
Hemoglobin (g/dL), mean ± SD	13.88 ± 1.65	13.46 ± 1.78	0.09
Leukocytes (x10^9), mean ± SD	9.68 ± 2.81	10.78 ± 2.44	0.008
Neutrophils /lymphocytes, mean ± SD	2.57 ± 0.99	3.03 ± 0.92	0.002
LVEF (%), mean ± SD	44.92 ± 8.99	36.84 ± 9.28	0.001

Note: Clinical and biochemical parameters were defined on patient admission.

NSTEMI – non-ST-elevation myocardial infarction; STEMI – ST-elevation myocardial infarction; GERBI – gastroesophageal reflux disease; COPD – chronic obstructive pulmonary disease; CRP – C-reactive protein; LVEF – left ventricular ejection fraction; SD – standard deviation.

coronary artery disease, longer time from onset of pain to PCI, and longer hospitalization, had higher adverse outcomes, and a statistically significant difference was observed in monitoring the same parameters in the group of patients without MACE. Moreover, statistical significance was observed by monitoring the values of troponin and left ventricle ejection fraction (LVEF), C-reactive protein (CRP), glycemia, and leukocytes, with a higher prevalence in patients with MACE, compared to the group of patients without MACE.

The mean time to onset of MACE in our patients diagnosed with AMI treated with PCI during the one-year follow-up was 334.4 ± 4.3 days. Statistical significance was observed in patients with STEMI in whom the mean time to onset of MACE was shorter (329.0 ± 5.5 days) compared with patients with NSTEMI (346.1 ± 6.2 days) (Log Rank – Mantel-Cox: χ^2 = 5.005, p = 0.02; Breslow: χ^2 = 4.9, p = 0.03; Tarone-Ware: χ^2 = 4.9, p = 0.03) (Figure 2).

Patients recently classified as high-risk of developing MACE based on the values of the GRACE, SYNTAX I, and SYNTAX II scores had significantly more often MACE compared to patients who had a low or moderate risk of developing MACE and those who did not have MACE (Table 2).

In order to analyze the time predictor until the occurrence of the MACE, the COX regression analysis (Enter method) was used (Table 3). Three models (Basic, Score, and Comprehensive model) were created, which included all variables that showed a statistically significant difference in frequency in patients with and without MACE.

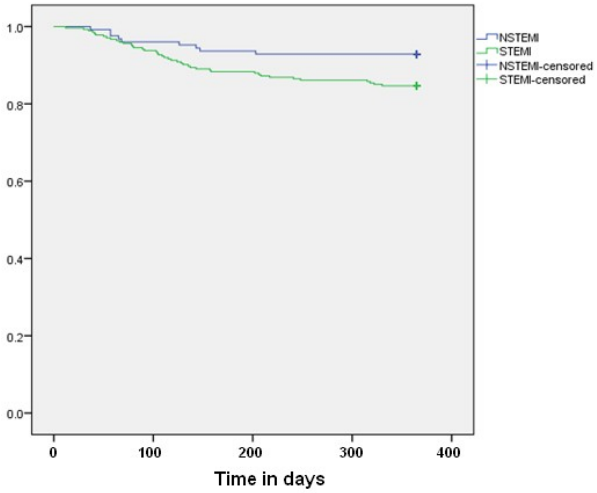

Fig. 2 – Kaplan-Mayer curve of time to onset of major adverse cardiovascular events (MACE) in patients with myocardial infarction treated with percutaneous coronary intervention (PCI) for a period of one year. NSTEMI – non-ST-elevation myocardial infarction; STEMI – ST-elevation myocardial infarction.

Table 2

Differences in values of the GRACE, SYNTAX I, and SYNTAX II scores between patients who developed major adverse cardiovascular events (MACE) during the one-year follow-up and those who did not

Variables	MACE		*p*
	no	yes	
SYNTAX I score, n (%)			
low risk	169 (99.4)	1 (0.6)	
moderate risk	161 (85.6)	27 (14.4)	0.001
high risk	19 (45.2)	23 (54.8)	
SYNTAX II score, n (%)			
low risk	108 (100.0)	0 (0.0	
moderate risk	189 (95.5)	9 (4.5)	0.001
high risk	52 (55.3)	42 (44.7)	
GRACE score, n (%)			
low risk	141 (93.4)	10 (6.6)	0.001
moderate risk	163 (90.6)	17 (9.4)	
high risk	45 (65.2)	24 (34.8)	
SYNTAX I score, mean ± SD	23.37 ± 4.85	31.11 ± 3.32	0.001
SYNTAX II score, mean ± SD	25.04 ± 4.89	32.87 ± 3.08	0.001
GRACE score, mean ± SD	115.78 ± 19.99	133.51 ± 22.73	0.001

GRACE – Global Registry of Acute Coronary Events; SYNTAX – Synergy between PCI (percutaneous coronary intervention) with Taxus and Cardiac Surgery.

All models showed statistical significance (p = 0.001) in examining the prediction of time to onset of MACE.

In the COX regression analysis in the first "Baseline Model", the NSTEMI entity was a significant predictor of the longer time to the onset of MACE. After the inclusion of the GRACE, SYNTAX I, and SYNTAX II scores in the next "Score Model", the NSTEMI entity lost significance in predicting the time to MACE, and only the SYNTAX II score remains a significant predictor of time to MACE onset (Table 3).

Namely, with each increase in the SYNTAX II score by one unit, the risk of shortening the time to the onset of MACE increases by 52.7%.

In the "Comprehensive Model", a higher SYNTAX II score, a long time from disease onset to PCI [95% confidence interval (CI): 1.000−1.017; p = 0.039], and higher age (95% CI: 0.907−0.994; p = 0.027) were the only risk factors that affect the shortening of the time to MACE occurrence (Table 3).

The significance of all three models in the prediction of the occurrence of MACE in the patients of our study was also confirmed in the logistic regression analysis (Enter method): ["GRACE model", χ^2 = 114.8; p = 0.001; B = -1.9; Wald = 164.6; Exp (B) = R2 Nagelkerke = 0.5; classification % = 90.8; "SYNTAX I model", χ^2 = 135.3; p = 0.001; B = -1.9; Wald = 164.6; Exp (B) = 0.1; R^2 Nagelkerke = 0.5; classification % = 89.8; "SYNTAX II model", χ^2 = 143.03; p = 0.001; B = -1.9; Wald = 164.6; Exp (B) = 0.1; R^2 Nagelkerke = 0.6; classification].

To assess the validity of the prediction of the occurrence of MACE using the GRACE, SYNTAX I, and SYNTAX II scores, ROC analysis was used (Figure 3).

Table 3

COX regression analysis of predictors of time of occurrence of major adverse cardiovascular events (MACE)

Variables	Basic model			Score model			Comprehensive model		
	Exp (B)	95% CI	*p*	Exp (B)	95% CI	*p*	Exp (B)	95% CI	*p*
Myocardial infarction entity (NSTEMI/STEMI)	0.449	0.219; 0.923	0.029	1.158	0.528; 2.538	0.715	1.236	0.515; 2.965	0.635
GRACE score				1.001	0.984; 1.020	0.878	0.992	0.968; 1.016	0.496
SYNTAX I score				0.950	0.778; 1.160	0.616	0.887	0.687; 1.146	0.359
SYNTAX II score				1.527	1.208; 1.930	0.001	1.527	1.140; 2.043	0.004
Age							0.949	0.907; 0.994	0.027
Time from disease onset to PCI							1.009	1.000; 1.017	0.039
χ^2/ p (the whole model)	5.003/ 0.025			104,125/ 0.001			162,254/ 0.001		
χ^2/ p (changes from the previous model)	5.572/ 0.018			106,684/ 0.001			25,387/ 0.321		

NSTEMI – non-ST-elevation myocardial infarction; STEMI – ST-elevation myocardial infarction; PCI – percutaneous coronary intervention; GRACE – Global Registry of Acute Coronary Events; SYNTAX – Synergy between PCI with Taxus and Cardiac Surgery.

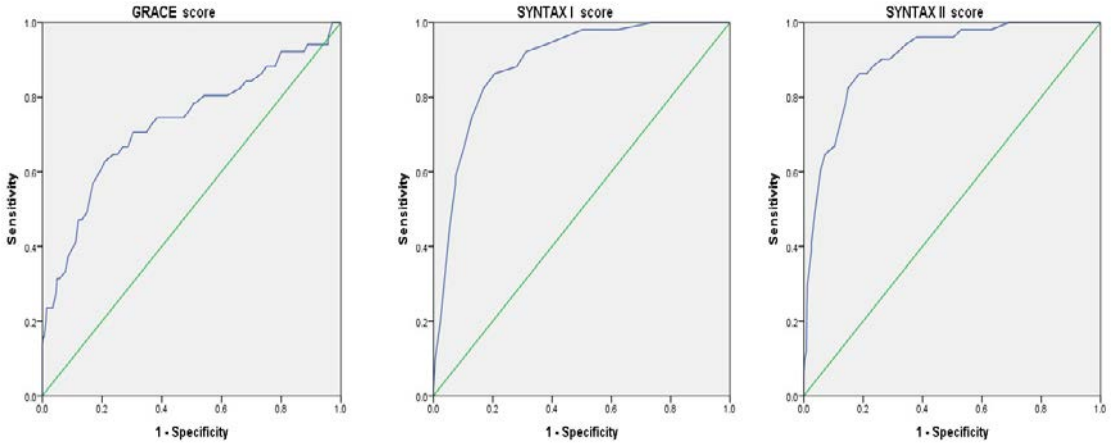

Fig. 3 – Receiver Operating Characteristic (ROC) curve of prediction of major adverse cardiovascular events (MACE) by the GRACE, SYNTAX I and SYNTAX II scores. GRACE – Global Registry of Acute Coronary Events; SYNTAX – Synergy between PCI (percutaneous coronary intervention) with Taxus and Cardiac Surgery.

According to the ROC analysis, the GRACE score explained the occurrence of MACE in 73.0% of patients in our study [AUC (area under curve) = 0.7; 95% CI 0.644 – 0.8; p = 0.001]. The GRACE score of 123.5 was identified as the optimal value for predicting the occurrence of MACE in our patients, with a sensitivity of 74.5% and a specificity of 61.6% (Figure 3).

The SYNTAX I score accurately explained up to 89.1% (AUC = 0.9; 95% CI 0.8–0.9; p = 0.001) of the occurrence of MACE in our patients. The SYNTAX I score of almost 27.5 was identified as the optimal value for predicting the occurrence of MACE in our patients, with a sensitivity of 86.3% and a specificity of 79.4% (Figure 3).

Finally, according to the ROC analysis, the SYNTAX II score explained the occurrence of MACE in 90.5% of the patients in our study (AUC = 0.9; 95% CI 0.9; p = 0.001). The SYNTAX II score of 29.3 was identified as the optimal value for predicting the occurrence of MACE in our patients, with a sensitivity of 88.2% and a specificity of 76.8% (Figure 3).

Discussion

Patients hospitalized for AMI, depending on the severity of the disease, show different values of clinical and laboratory characteristics.

Determining the severity of coronary artery disease and risk stratification is essential for choosing the right therapeutic approach. The GRACE score is a strong, independent predictor of MACE in patients with AMI [20] but is not optimized for patients with PCI due to the lack of angiographic findings in their scoring system, which is consistent with the results of our study.

Moreover, we observed that patients with MACE had statistically significantly higher values of the GRACE, SYNTAX I, and SYNTAX II scores compared with the group of patients who did not have MACE.

In order to analyze the predictors of time to the occurrence of MACE, regression analysis (Enter Method) was used. Three models (Baseline, Score, and Comprehensive models) were created that included all variables that showed a statistically significant difference in incidence in patients with and without MACE. All models showed statistical significance (p = 0.001) in examining the prediction of time to onset of MACE. In general, studies indicate a reduced prognostic value of the GRACE score as a useful tool for the initial classification of patients with NSTEMI – AMI but not for patients with PCI due to lack of angiographic findings in the scoring system, giving preference to the SYNTAX II score [21].

By regression analysis in the first “Basic model”, in NSTEMI entity, we noticed the significance of all three scores as predictors of a long time to the appearance of MACE. After the inclusion of the GRACE, SYNTAX I, and SYNTAX II scores in the next “Score Model”, the NSTEMI entity loses significance in predicting the time to MACE, and only the SYNTAX II score remains as a significant predictor of time to MACE occurrence.

Namely, with each increase in the SYNTAX II score by one unit, the risk of shortening the time to the onset of MACE increases by 52.7%.

In order to analyze the GRACE score as a predictor of the SYNTAX I and SYNTAX II scores, linear regression was used, and all variables that showed a statistically significant correlation with the examined scores were analyzed. All models showed statistical significance (p = 0.001) in the study of assessing the efficiency of predicting the MACE by the SYNTAX I and SYNTAX II scores.

In the regression analysis of the GRACE score as a predictor of SYNTAX I score, it was noticed that a higher GRACE score is a significant predictor of higher values of SYNTAX I score in the whole sample of patients diagnosed with myocardial infarction treated with PCI, in patients with STEMI entity, and in the group of patients with NSTEMI entity. Another study [22] presented the GRACE score as acceptable in the clinical risk stratification of patients with NSTEMI in different age groups. A study conducted in Brazil found that the GRACE score has 50% sensitivity and 98% specificity for predicting a high risk of death in patients with NSTEMI [23].

In addition to the GRACE score, significant predictors of the SYNTAX I score, both in the whole sample and in patients with NSTEMI and STEMI, were a higher number of diseased coronary arteries and the presence of peripheral arterial disease. In addition, a significant predictor of higher SYNTAX I scores in the entire sample of patients diagnosed with myocardial infarction treated with PCI was a long time from the onset of pain to PCI.

Some researchers evaluating the accuracy of the GRACE assessment in predicting the severity and degree of coronary artery stenosis in the correlation with the SYNTAX score found a sensitivity of 73.5% and a specificity of 60% of the GRACE score in predicting coronary artery stenosis in patients with ACS [23, 24].

Analysis of the SYNTAX II score showed that the higher GRACE score is a significant predictor of higher SYNTAX II score values in the entire sample of patients diagnosed with myocardial infarction treated with PCI, as well as in patients with both entities of myocardial infarction. In addition to the GRACE score, significant predictors of the SYNTAX II score in all three models (whole sample, NSTEMI, and STEMI) were a higher number of diseased coronary arteries, the presence of peripheral arterial disease, and lower LVEF values.

In addition, as a significant predictor of higher SYNTAX II scores in the entire sample of patients diagnosed with PCI-treated myocardial infarction, there was a long time from pain to PCI, while in patients with NSTEMI significant predictors of higher SYNTAX II score were lower LVEF and higher HbA1c values.

In the logistic regression analysis in the first GRACE model, the score was not a statistically significant predictor of the occurrence of MACE in patients diagnosed with AMI-treated PCI. Significant risk factors for the occurrence of MACE in the GRACE model were younger age and longer

hospitalizations, while the protective factor was a smaller number of diseased coronary arteries.

In the SYNTAX I model, significant risk factors for the occurrence of MACE were higher SYNTAX I score, younger age, and the presence of hypertension.

In the SYNTAX II model, significant risk factors for the occurrence of MACE were higher SYNTAX II scores and younger life expectancy.

According to the ROC analysis of the value of scores in the prediction of MACE, the sensitivity for the GRACE score was 74.5% and specificity 61.6%; for the SYNTAX I score, sensitivity was 86.3% and specificity 79.4%; for the SYNTAX II score, sensitivity was 88.2% and specificity 76.8%.

The use of clinical and angiographic risk scores in clinical practice in patients with AMI and PCI has provided a powerful clinical stratification tool that can predict MACE more accurately than by applying these scores individually in order to select the best treatment strategy and improve prognosis and the outcome of AMI.

Conclusion

The results of the study indicate that the combination of clinical and anatomical variables in the GRACE and SYNTAX I scores is useful for predicting MACE during hospitalization, but that the SYNTAX II score allows more accurate and individualized mortality assessment over a period of one year after hospitalization, and is, therefore, a clinically more useful tool for predicting MACE occurrence.

Conflict of interest

The authors declare no conflict of interest.

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Influence of riboflavin and ultraviolet-light treatment on plasma proteins – protein S and alpha 2-antiplasmin – in relation to the time of administration

Uticaj riboflavina i ultravioletnog zračenja na proteine plazme – protein S i alfa 2-antiplazmin – u odnosu na vreme primene

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Abstract

Background/Aim. After the introduction of a careful selection procedure for blood donors and the implementation of highly sensitive screening tests for transfusion-transmitted infections (TTIs), blood has become a very safe product concerning TTIs. However, due to the existence of a "window" period during which these "markers" cannot be detected, as well as the emergence of new pathogens, the risk is still present. Implementation of pathogen reduction technology (PRT) provides a proactive approach to improving blood safety. By damaging nucleic acids, PRT selectively inactivates pathogens and leucocytes. Nevertheless, during the process, plasma proteins are also damaged to some extent. The aim of this study was to conclude whether there is a difference in the effect of PRT on protein S (PS) and alpha 2-antiplasmin (α2AP) regarding the time of inactivation: inactivation immediately after plasma separation from whole blood (before freezing) vs. inactivation after freezing/thawing. **Methods.** The voluntary donors' blood is taken into a quadruple bag system, centrifuged, and separated into blood products. Control group plasma was first inactivated by the Mirasol® PRT system and then frozen. Experimental group plasma was immediately frozen and, after four months, thawed and inactivated. PS and α2AP activity was examined in samples after separation, inactivation, and thawing. **Results.** Analyzing PS and α2AP activity, no statistically significant difference was found between the initial samples. The trend of protein activity reduction after inactivation and freezing/thawing was present in both groups but without a statistically significant intergroup difference. **Conclusion.** No statistically significant difference was found between the activity values of PS and α2AP after immediate inactivation, before freezing, and after freezing/thawing, making stored plasma units suitable for safe and efficient inactivation directly before clinical use and according to the patient's blood type.

Key words:
alpha-2-antiplasmin; plasma; proteins; protein s; riboflavin; safety; time factors; ultraviolet rays.

Apstrakt

Uvod/Cilj. Pažljivim izborom davalaca i korišćenjem visoko osetljivih „skrining" testova za detekciju uzročnika infekcija koje se mogu preneti putem transfuzije (TTI), krv je postala veoma bezbedan produkt u odnosu TTI. Međutim, zbog postojanja perioda „prozora" tokom kojeg se ovi „markeri" ne mogu detektovati, kao i pojave novih patogena, rizik je i dalje prisutan. Uvođenjem tehnologije za redukciju patogena (PRT) ostvaruje se proaktivan pristup u poboljšanju bezbednosti krvi. Oštećenjem nukleinskih kiselina, PRT selektivno inaktivira patogene i leukocite. Nažalost, tokom ovog procesa se u određenom stepenu oštećuju i proteini plazme. Cilj rada je bio da se utvrdi postojanje razlika u efektu PRT na protein S (PS) i α2-antiplazmin (α2AP) u zavisnosti od vremena inaktivacije: ukoliko se plazma inaktivira odmah po izdvajanju iz jedinice krvi (pre zamrzavanja) ili ako se inaktivira naknadno, posle zamrzavanja/odmrzavanja. **Metode.** Krv dobrovoljnih davalaca je bila prikupljena u sistem četvorostrukih kesa, centrifugirana i razdvojena na produkte. Kontrolna plazma je bila najpre inaktivisana

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Mirasol® PRT sistemom i, potom, zamrznuta. Plazma eksperimentalne grupe je bila odmah zamrznuta, a nakon četiri meseca odmrznuta i inaktivisana. Aktivnost PS i α2AP je bila ispitivana u uzorcima plazme posle separacije, inaktivacije i odmrzavanja. **Rezultati.** Analizom rezultata aktivnosti PS i α2AP utvrđeno je da nije bilo statistički značajne razlike između inicijalnih uzoraka. Nakon inaktivacije i zamrzavanja/odmrzavanja postojao je trend pada aktivnosti ovih proteina u obe grupe, ali statistički značajna razlika između kontrolne i eksperimentalne grupe nije ustanovljena. **Zaključak.** Nije postojala statistički značajna razlika između vrednosti aktivnosti PS i α2AP nakon inaktivacije pre zamrzavanja, odnosno posle zamrzavanja/odmrzavanja, što ukazuje da uskladištene jedinice plazme mogu biti sigurno i efikasno inaktivisane neposredno pre kliničke upotrebe, u skladu sa krvnom grupom primaoca.

Ključne reči:
alfa-2-antiplazmin; plazma; protein; protein s; riboflavin; bezbednost; vreme, faktor; ultravioletni zraci.

Introduction

Plasma is the liquid part of blood that contains pro and anticoagulant factors. Plasma can be defined as fresh frozen plasma (FFP) if frozen within 8 hours from collection, plasma 24 (frozen within 24 hours from collection), or thawed plasma. FFP and plasma 24 contain all coagulation factors. If FFP and plasma 24 are thawed, they become thawed plasma and can be stored at 4 °C for five days.

Plasma is used for treating multiple coagulation deficiencies that occur in patients with liver failure, vitamin K deficiency, warfarin overdose, disseminated intravascular coagulation, or massive transfusion. Sometimes, plasma can be used for treating patients with single factor deficiency, such as factor XI deficiency. FFP is used as a replacement fluid in therapeutic plasma exchange (TPE). In cases of thrombotic thrombocytopenic purpura (TTP), TPE removes inhibitors, and plasma provides a metalloprotease (ADAMTS13), thus reversing the symptoms [1, 2].

Protein S (PS) is a vitamin K-dependent protein that enhances the anticoagulant effect of activated protein C (APC). PS is synthesized in hepatocytes, endothelial cells, megakaryocytes, and brain cells. As a cofactor for APC, PS has a role in the inactivation of the factors Va and VIIIa. Factor Va inactivation happens as an ordered series of peptide bond cleavage in the molecule's heavy chain, first rapid cleavage at Arg 506, then slower cleavage at Arg 306, and then Arg 679. Interaction of PS and APC results in both an increased affinity for negatively charged phospholipids and a 20-fold enhancement of the slower phase of factor Va inactivation. Only 40% of plasma PS is free and available, whereas the rest is bound to C4b-binding protein and cannot interact with APC [3].

The primary inhibitor of plasmin synthesized in the liver is alpha 2-antiplasmin (α2AP). Bound plasmin digests clots and restores blood vessel lumen, and free plasmin in the circulation digests fibrinogen, factor V, factor VIII, and fibronectin which may cause potentially fatal primary fibrinolysis. α2AP rapidly and irreversibly binds free plasmin [4].

Blood for transfusion is extremely safe concerning virus transmission given the improved donor screening method and a range of assays for detecting antibodies, antigens, and genomes [5]. However, the emergence of new pathogens, such as West Nile virus, Severe Acute Respiratory Syndrome (SARS) virus, Chikungunya, Dengue, and many others, make a permanent threat [6, 7].

The other issue is the so-called "window period" during which detecting the presence of the pathogen is impossible, no matter how testing technologies are sensitive. In addition, bacterial contamination, especially of platelet concentrates, and the presence of protozoa transmitted by blood pose significant risks. The safety of the blood is also compromised by the presence of residual leukocytes that can be found even after leukoreduction. For all of the above, the implementation of pathogen reduction technology (PRT) provides a proactive technology approach to blood safety by inactivating pathogens possibly present in blood products [8–14].

The aim of this study was to compare the effects of PRT treatment on PS and α2AP in common prestorage versus post-storage inactivation (after freezing/thawing) setting. We expect that previously frozen plasma units can be inactivated without additional damage to PS and α2AP compared to immediately inactivated plasma, which will allow us to do this procedure before clinical use and according to the blood type needed.

Methods

Whole blood from random healthy donors aged 18 to 65 was collected into a quadruple blood bag system (Terumo, Japan) according to the manufacturer's instructions. Donors were tested for hepatitis B and C virus (HBV and HCV), human immunodeficiency virus (HIV), and lues markers by chemiluminescent immunoassay using Architect 2000 (Abbott, USA), as well as by polymerase chain raection (PCR) test (COBAS AmpliPrep/TaqMan, Roche, Germany).

Primary separation was performed 2–8 hrs after collection by "hard" spin: speed 3,603 rpm (3,890 g) for 10 min (radius: 268 mm, acceleration: 6, brake: 4) at 4 ± 2 °C. After separation, plasma units were: a) inactivated and frozen [prestorage setting or control group (CG); n = 30] or b) immediately frozen [post-storage setting or study group (SG); n = 30] at -80 ± 5 °C and stored at -40 ± 5 °C. After four months, plasma from the SG was thawed and inactivated, as was the plasma from the CG for sampling.

Plasma units were inactivated by the Mirasol® PRT system (Terumo BCT, USA) in the following way: plasma was transferred into an illumination bag, riboflavin (RB) 35 ± 5 mL, was added using the sterile connection (Sterile

Tubing Welder TSCD Terumo, Japan), residual air was extruded to an empty RB bag, and the set (Mirasol® PRT Plasma Illumination/Storage Set) was placed in the illuminator (Mirasol® PRT, Terumo BCT, USA). Plasma was then exposed to UV light (k = 265–370 nm) at the dose of 6.24 J/mL, with constant horizontal shaking (120 cycles/min).

In the prestorage setting (CG), plasma samples (8 mL) were taken immediately after separation (initial sample or autocontrol – AC_{CG}) and following PRT treatment (sample I – S-I_{CG}). Before testing, AC_{CG} samples were held at room temperature for a period equivalent to the illumination time of treated units. Inactivated plasma units were frozen and stored until thawing and testing (sample II – S-II_{CG}).

In the post-storage setting (SG), plasma samples were tested immediately after separation (initial sample or autocontrol – AC_{SG}), after freezing/thawing (sample I – S-I_{SG}), and following PRT treatment (sample II – S-II_{SG}). S-I_{SG} were maintained at room temperature (20 ± 2 °C) for a period of illumination of thawed plasma units.

Natural inhibitors, PS and α2AP, were determined by the BCS XP Coagulation system (Siemens, Germany).

Data for PS and α2AP activity were compared: initial vs. final (AC_{CG} vs. S-II_{CG} and AC_{SG} vs. S-II_{SG}) in both prestorage and post-storage settings, as well as the calculated recovery between the groups.

Descriptive data of plasma research were expressed as mean value ± standard deviation (SD) for each parameter. Statistical analyses were performed by comparing groups using a standard Student *t*-test for paired sample sets. Differences were considered statistically significant if the *p*-value was less than 0.05.

Results

In this study, we examined the influence of time of PRT treatment on PS and α2AP activity by comparing results obtained in prestorage (CG) and post-storage (SG) settings, i.e., immediate inactivation vs. inactivation after four months of cryostorage at -40 ± 4 °C/ thawing PRT application.

The data analysis of the two groups indicated no significant differences between initial samples – autocontrols (AC_{CG} vs. AC_{SG}) for these proteins. Under identical handling fashion, the inactivation process and freezing/thawing conditions of PS and α2AP result in comparable activities in both prestorage and post-storage PRT-treatment settings (Table 1).

The recovery of PS and α2AP was calculated as the ratio, expressed in percent, of the value after PRT treatment and freezing/thawing process compared to the corresponding initial level before *ex vivo* manipulation and was labeled "calculated recovery".

There was a trend toward reduction of protein activity in both prestorage and post-storage PRT-treatment samples (AC vs. S-II; $p < 0.05$).

The rate of recovery of PS was similar in the two groups: 94% recovery in prestorage vs. 90% in post-storage, just as was the recovery of α2AP, which was 69% in prestorage setting compared with 83% in post-storage settings making no significant difference of natural inhibitors activity between the two groups.

Discussion

Protein S is made up of 635 amino acid residues arranged in multiple domains. In human plasma, 60% of PS is bound to the complement regulatory protein C4b-binding protein (C4BP), and the remaining 40% is circulating free.

Protein S is primarily an anticoagulant protein but also has other important roles in immune and vascular systems. Anticoagulant functions of PS are the following: 1) cofactor to APC in the regulation of factor Va in prothrombinase complex and factor VIIIa in tenase complex; 2) direct APC-independent inhibition of prothrombinase and tenase complexes; 3) cofactor to tissue factor pathway inhibitor alpha (TFPIα) in inhibition of factor Xa [15].

Protein S deficiency leads to the risk of venous thrombosis but could be as well associated with arterial thrombotic events [16].

Human α2AP circulates in the blood as a single chain glycoprotein. The protein regulates fibrinolysis in three ways: by forming a complex with plasmin, by inhibiting plasminogen from adsorbing to fibrin, and by making fibrin more resistant to local plasmin (through cross-linking via factor XIIIa). Both thrombus associated and plasma α2AP regulate fibrinolysis, rapidly inactivating plasmin and

Table 1

Pathogen reduction technology (PRT)-treated fresh frozen plasma (FFP) evaluation

Plasma proteins	Prestorage treatment (control group)			Post-storage treatment (study group)		
	AC_{CG}	S-I_{CG}[a]	S-II_{CG}[a]	AC_{SG}	S-I_{SG}	S-II_{SG}[a]
PS	1.30 ± 0.00	1.26 ± 0.16	1.20 ± 0.26	1.27 ± 0.09	1.32 ± 0.24	1.15 ± 0.21[b,c]
α2AP	1.09 ± 0.07	1.02 ± 0.15	0.81 ± 0.15[b,c]	1.06 ± 0.06	1.08 ± 0.16	0.88 ± 0.08[c]

CG – prestorage setting, control group: AC_{CG} – autocontrol, initial sample taken immediately after separation of blood plasma; S-I_{CG} – first sample, taken immediately following the PRT treatment of separated blood plasma; S-II_{CG} – second sample, blood plasma was PRT-treated, and afterward frozen, stored, and then thawed.

SG – post-storage setting, study group; AC_{SG} – autocontrol, initial sample taken immediately after separation of blood plasma; S-I_{SG} – first sample, separated blood plasma was frozen, stored, thawed, and PRT-treated afterward; PS – protein S; α2AP – alpha2-antiplasmin.

[a] – Riboflavin (RB)-associated dilution factor implied.

[b] – AC vs. S-II ($p < 0.05$).

[c] – S-I vs. S-II ($p < 0.05$).

forming stable inactive complex plasmin-α2AP. In α2AP deficiency, bleeding is caused by premature dissolution of hemostatic plugs before tissue and vessel reparation are finished. Therefore, bleeding is often delayed after trauma or invasive procedures [4].

Acquired deficiency of α2AP may occur in patients with severe liver illness when plasma levels fall as low as 8%. Sometimes it is also seen in patients with renal disease, disseminated intravascular coagulation, and patients on thrombolytic therapy [17, 18].

A lot of measures have been introduced to prevent the transmission of infectious agents through blood, so the risk of classical TTI agents (HBV, HCV, and HIV) has been drastically reduced. Unfortunately, blood transfusion still constitutes a risk because of a "window period", new emerging pathogens, parasites, and bacteria [19]. Therefore, a much better and more efficient option would be a preemptive approach, which includes PRT.

Pathogen reduction effectively inactivates most clinically relevant viruses (RNA or DNA, single or double-stranded, enveloped or nonenveloped, intracellular or extracellular). Furthermore, it inactivates gram-positive and gram-negative bacteria, spirochetes, Rickettsia and protozoa, and lymphocytes and probably protects against pathogenic agents that will emerge in the future. Bad sides of PRT are decreased yield for some products (especially platelets), insufficient reduction of some high-titer, nonenveloped agents (hepatitis A virus, parvovirus B-19), concern for potential toxicity, no single pathogen reduction (PR) system for all blood products at present, and anticipated high cost [20].

Mirasol® PRT system uses water-soluble vitamin B2, RB, and UV light. RB is rapidly excreted and cannot be stored in the body. RB is a photosensitizer and mediates selective damage to nucleic acids after exposure to light [21]. RB attaches to nucleic acids and mediates an oxygen-independent electron transfer that causes modification of nucleic acids, mostly guanine, while RB is converted into his photoproduct lumichrome [22]. Damage induced by RB is irreversible because replication and repair processes are diminished due to guanine base modification [23, 24].

Blood safety in terms of TTIs is of particular importance for vulnerable groups of patients who are either exposed to a large number of chemoproducts for a short time or are immunocompromised due to the therapy they receive [patients with thrombotic thrombocytopenic purpura (TTP) or liver transplant recipients]. Due to the aforementioned, it is necessary to have a sufficient amount of PRT-treated FFP at all times, which is not rational, bearing in mind that FFP is given according to the blood type of patients and that universal "AB" FFP is not sufficient since it is the rarest blood type. In addition, it is not realistic to expect that universal inactivation of blood products will soon enter into routine practice due to the cost and complexity of the procedure (but over time, it will prove cost-neutral and possibly cost-saving). The ideal solution would be to inactivate the required amount of stored FFP of appropriate blood groups for a particular patient immediately before administration.

The aim of this study was to show that subsequently - post-storage (after freezing/thawing) treated FFP has the same quality in terms of natural coagulation inhibitors as prestorage, "classically" treated FFP [25].

Data obtained in this study, as those from the previous related study [26], analyzed the plasma hemostatic activity before and after PRT treatment and cryostorage in both prestorage and post-storage settings. As reported, PRT-treated plasma demonstrates a reduction in plasma procoagulant factors [13, 27–31]. This reduction in activity is noted immediately after prestorage PRT treatment and remained relatively constant during cryostorage from 75–79% (for FVIII) to 80–87% (for FII). In our previous study, procoagulant activities are expressed as relative numbers [26] before and after PRT treatment in both prestorage and post-storage settings. The calculated recovery for different procoagulant factors was similar in the two groups: for FII, 79% in CG vs. 81% in SG; for FV, 71% in CG vs. 88% in SG; for FVII, 75% in CG vs. 83% in SG; for FVIII, 70% in CG vs. 71% in SG; for FIX, 77% in CG vs. 72% in SG; and for FX, 75% in CG vs. 65% in SG [26]. Results obtained were comparable with data from the literature [13, 27–31].

For natural inhibitors, activities of PS were similar (no statistical significance) in both groups: the calculated recoveries in prestorage and post-storage groups were 94% and 90%, respectively. In our previous study, the calculated recovery of protein C was 84% in the prestorage and 86% in the post-storage group [26]. Moreover, recovery of α2AP activity of 69% vs. 83% in prestorage and post-storage groups, respectively, was not statistically significant. Similar results were obtained by Singh et al. [32] with amotosalen and UV light, where retention of inhibitors was 78% to 98%, while Smith and Rock [27] had retention between 91% and 100% with Mirasol® PRT.

However, the activity of AT-III was significantly higher ($p < 0.05$) after post-storage PRT treatment [26].

Conclusion

This study, similar to our previous study concerning plasma constituent integrity, confirmed that no clinically relevant intergroup differences (prestorage vs. post-storage PRT treatment) in plasma constituents levels were observed. After post-storage treatment, proteins, quantity, and activity in FFP continue to be satisfying and can be used in clinical practice. Even more, the recovery obtained for AT-III in the post-storage setting was higher. Thus, previously cryostored FFP units could be safely and effectively inactivated just before their clinical application, which is of great importance because only necessary plasma units will be inactivated instead of random ones. In that manner, both significant financial resources and the time for preparation will be saved.

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Hygiene status of food contact surfaces in public school canteens in the city of Novi Sad, Serbia

Higijenski status kontaktnih površina pri rukovanju hranom u kantinama državnih škola grada Novog Sada, Srbija

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Abstract

Background/Aim. Establishing a food safety control system is extremely important in preventing diseases associated with foodborne pathogens. The aim of this study was to examine the hygiene status of food contact surfaces and the application of good hygiene practices by food handlers in school canteens. **Methods.** A total of 10,366 swabs were taken from food contact surfaces, including food handler's hands from public school canteens in Novi Sad, Serbia, over ten years (2008–2017), covering voluntary good hygiene practices and obligatory Hazard Analysis Critical Control Points (HACCP) implementation periods. **Results.** Statistically significant differences ($p < 0.05$) in aerobic colony counts (ACCs) on surfaces between two examined periods were found. A general positive trend regarding the reduction of microbial contamination of food contact surfaces was observed. The percentage of surfaces swabs with ACCs above 2.48 log CFU/cm^2 significantly decreased ($R^2 = 0.453$) during the study period, and the percentage of coagulase-positive *Staphylococcus* in the swabs also decreased, but not significantly ($R^2 = 0.264$), and average annual *Enterobacteriaceae* counts above the established limit values on surfaces significantly decreased ($R^2 = 0.442$) over the years. A significantly higher ($p < 0.05$) percentage of workers' hands harbored ACCs, coagulase-positive *Staphylococcus,* and *Enterobacteriaceae* above the established limits than the equipment or work surfaces. **Conclusion.** The results obtained showed the proper implementation of good hygiene practices concerning food contact surfaces, but the implementation of good personal hygiene practices needs enhanced supervision.

Key words:
enterobacteriae; hygiene; public health; school; serbia; surface properties; staphylococcus.

Apstrakt

Uvod/Cilj. Uspostavljanje sistema kontrole bezbednosti je izuzetno važno u prevenciji bolesti povezanih sa patogenima koje se prenose hranom. Cilj ovog istraživanja bio je da se utvrdi higijenski status kontaktnih površina i primena dobre higijenske prakse pri rukovanju hranom u školskim kantinama. **Metode.** Ukupno 10 366 briseva uzeto je sa površina koje dolaze u kontakt s hranom i ruku osoblja zaposlenog u školskim kantinama u Novom Sadu u desetogodišnjem periodu (2008–2017). Istraživanje je uključivalo dva perioda: a) dobrovoljnu primenu dobre higijenske prakse i b) obaveznu implementaciju sistema *Hazard Analysis Critical Control Points* (HACCP). **Rezultati.** Statistički značajna razlika ($p < 0,05$) utvrđena je u ukupnom broju aerobnih bakterija (*aerobic colony counts* -ACCs) na površinama. Primećen je opšti pozitivni trend u pogledu smanjenja mikrobiološke kontaminacije površina koje dolaze u kontakt s namirnicama. Učestalost nalaza briseva sa utvrđenim ukupnim ACCs iznad 2,48 log CFU/cm^2 smanjivala se statistički značajno ($R^2 = 0,453$) tokom perioda ispitivanja; učestalost prisustva koagulaza pozitivnih stafilokoka se takođe smanjivala, ali ne statistički značajno ($R^2 = 0,264$), dok se prosečan broj enterobakterija iznad utvrđenih graničnih vrednosti na površinama statistički značajno smanjio tokom godina ($R^2 = 0,442$). Na brisevima ruku ispitanog osoblja utvrđena je statistički značajno viša ($p < 0,05$) učestalost prisustva ukupnog ACCs, koagulaza pozitivnih stafilokoka i enterobakterija iznad utvrđenih granica u odnosu na briseve uzete sa opreme ili radnih površina. **Zaključak.** Dobijeni rezultati pokazuju pravilnu primenu dobre higijenske prakse kada su u pitanju površine koje dolaze u kontakt s hranom, dok implementacija prakse dobre lične higijene zaposlenih zahteva pojačan nadzor.

Ključne reči:
enterobacteriae; higijena; zdravstvena zaštita; škola; srbija; površina, svojstva; staphylococcus.

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Introduction

Food safety is one of the main public health challenges. Establishing a food safety control system is essential in preventing diseases associated with foodborne pathogens [1–2]. Along with the elderly and immunocompromised, young children fall in the population groups that are very susceptible to foodborne diseases [3]. School canteens must provide a high level of food safety and personal hygiene to ensure that all food is safe to eat. That implies that all food handlers must practice a high level of personal and food hygiene, including efficient hygiene of all food contact surfaces (FCS), knowing how to store foods at suitable and safe temperatures, and how to maintain cleanliness and prevent cross-contamination [4–5]. Food safety in canteens can be achieved through the application of good hygiene practice (GHP) during food preparation and distribution or the implementation of the Hazard Analysis Critical Control Points (HACCP) system [6–7].

Pathogenic microorganisms, which have the potential to cause food spoilage and food poisoning, are always present in food handling environments. Microorganisms are usually introduced into the food environment through raw materials, water, and employees [1, 8]. Sometimes, the application of good sanitation practices can prevent the growth of these organisms. However, if contamination levels are high or sanitation procedures are inadequate, transient microorganisms can become established, multiply, and become resident [2, 9–10].

The modern approach to food safety also includes complete control of the production process along the entire production chain, from farm to fork [11]. Food safety and quality policy is a set of regulations designed to result in food that will not endanger the health of consumers due to the presence of biological, chemical, or physical hazards above prescribed levels (for some of them, there is zero tolerance) [12, 13]. Numerous bacterial pathogens, such as *Escherichia (E) coli*, *Clostridium difficile*, and *Shigella* spp., can survive for months on dry surfaces and even longer on wet surfaces [14]. There is no doubt that standardization of food products facilitates food trade, and that applies to national and regional specialties, as well as to ready-to-eat foods. However, the fact is that all food intended for human consumption must be safe for human health [4].

Microbial analyses are an essential part of food safety practice and a tool to verify correct system function. Microbiological analysis of FCSs and estimation of the overall number of bacteria are essential in assessing and monitoring general hygiene.

A good food safety monitoring system in public school facilities (including preschool facilities) has been established in the City of Novi Sad, Serbia. Over the past decade, only one foodborne outbreak was recorded in a public kindergarten in Novi Sad. This outbreak was of histamine intoxication from fish and fish products [15].

The aim of this study was to examine the hygiene status of FCSs and to follow the trend in the change of an unacceptable number of the examined group of bacteria in ten years in public school canteens in the City of Novi Sad, Serbia, covering voluntary GHP and obligatory HACCP implementation periods.

Methods

Sampling

There is an annual food safety monitoring program in school canteens adopted and implemented by the Institute of Public Health of Vojvodina, Novi Sad, Serbia. The program provides microbiological testing of FCSs by taking swabs from equipment, surfaces, and hands of the kitchen staff from more than 100 public school food premises that prepare and/or distribute food, during a ten-year period, in the city of Novi Sad, Serbia.

Sampling was performed by trained personnel who completed the swab sampling training program. Each swab was moistened with sterile 1% buffered peptone water (10 mL in a tube), then leaned on the wall of the tube to drain excess liquid. Swabs were swiped on the surfaces delineated by a sterile template (10 × 10 cm), left to right and right to left, five times, and up and down five times. The swabs were returned to the tube aseptically, breaking the stick, and were transported to the laboratory in a cooler box at a maximum temperature of 4 °C. In order to check the hygiene status of FCSs and the implementation of GHP by food handlers over time, a total of 10,366 swabs were taken.

Microbiological methods

To verify the level of contamination, 4–5 samples were taken from each selected canteen twice a year, using the swab technique according to the International System for Standardization (ISO) 18593 standard [16]. For each facility, representative swab samples were taken from different FCSs and classified into three categories: equipment, countertops/working surfaces, and hands. Microbiological analyses included aerobic colony count (ACC), *Enterobacteriaceae* count, and counts of coagulase-positive *Staphylococcus*. All microbial analyses were conducted in an ISO/International Electrotechnical Commission (IEC) 17025:2005 accredited laboratory (Institute of Public Health of Vojvodina, Center for Hygiene and Human Ecology/Department for Sanitary Bacteriology, Novi Sad, Serbia).

Two periods were covered during the ten-year investigation period – before and after mandatory HACCP implementation (effective from May 31, 2011, with practical application started in 2012). Different methods were used for the microbiological analysis of swabs in these two periods. Until 2012 (the first period of investigation), swabs were analyzed referring to an internal guidance document adopted by the Institute of Public Health of Vojvodina, with established ACC limit values based on achieved GHP performed on the premises over a long period. From 2012 and on, after obligatory HACCP implementation, all swabs were analyzed according to ISO for ACC [17], *Enterobacteriaceae* [18–19], and coagulase-positive *Staphylococcus* counts [20, 21]. After appropriate storage and transport, a microbiological analysis should start as soon as possible, no later than four hours after sampling. Bacterial counts recovered from swabs taken from surfaces of defined size enclosed by templates were expressed as colony forming unit (CFU)/cm^2, while counts recovered from indeterminate surfaces (small parts of machines and apparatus used in food preparation, curved surfaces, hands,

etc.) were expressed as CFU/mL. All bacterial counts were transformed into logarithms. The microbiological criteria specific to the type of FCS were identical before and after HACCP implementation. The unacceptable number of ACCs on smooth surfaces – fine porcelain, glass, stainless steel, and metal is > 1 log CFU/cm^2; for other wooden, plastic, and stone surfaces > 1.48 log CFU/cm^2; for equipment and dishes – cutlery, plates, pots, etc. > 2 log CFU/cm^2; for bottles and other containers for liquid products > 0.3 log CFU/cm^2. The microbiological criterion for an unacceptable number of ACCs for food handlers' hands is > 2.30 log CFU/cm^2. Limit value for *Enterobacteriaceae* count, for all types of FCSs, is > 0.3 log CFU/cm^2 [22].

To assess the effectiveness of applied hygiene practices in school kitchen canteens, we divided the established number of ACC into four classes: Class I (≤ 2.10 log CFU/cm^2 or CFU/mL); Class II (results between 2.11 and 2.30 log CFU/cm^2 or CFU/mL); Class III (results between 2.31 and 2.48 log CFU/cm^2 or CFU/mL); and Class IV (> 2.48 log CFU/cm^2 or CFU/mL). Class I indicates a good standard of applied GHP, Classes II and III correspond to the satisfactory application of GHP, and Class IV represents unsatisfactory hygiene practice.

Statistical analysis

The data were first analyzed using descriptive statistics and presented as frequency and percentage (%) for categorical data. For numerical data, bacterial counts were expressed as mean ± standard error (SE). For statistical analysis, all bacterial counts were transformed into logarithms. Statistical analysis of the results was conducted using Microsoft Excel 2010 and GraphPad Prism software, version 7.00 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). The average total ACC was compared to the average ACC for each year individually by one-factor analysis of variance (ANOVA), while Student's t-test was performed to compare the average colony count separately. Statistical significance was at the level of $p < 0.05$. The coefficient of determination (R^2) was used to evaluate the significance of the bacterial count trends for ACC, coagulase-positive *Staphylococcus*, and *Enterobacteriaceae* above the established limits between 2008 and 2017 (Microsoft Office, Excel, 2010). Trends with R^2 values exceeding 0.30 were considered significant [23]. The χ^2 test was used to compare the percentages of incidence of ACC, coagulase-positive *Staphylococcus*, and *Enterobacteriaceae* on the examined surfaces.

Results

The total number of swabs in the four ACC classes over ten years is shown in Table 1. Table 1 shows the period before and after the implementation of HACCP. The incidence of Class IV findings was 10.86% in the first study period and 7.24% in the second. In all observed ACC classes, the prevalence of this type of bacteria was higher in the first study period.

Table 2 shows that the highest average ACC during the

Table 1

Number (percentage) of surfaces from school food preparation facilities with ACCs categorized in four classes, before and after mandatory HACCP implementation

Class	Obligatory HACCP (years)					Mandatory HACCP (years)						
	2008	2009	2010	2011	Σ	2012	2013	2014	2015	2016	2017	Σ
I	508 (80.95)	1,003 (81.55)	981 (79.78)	1,049 (84.62)	3,541 (81.83)	1,067 (87.46)	1,007 (82.54)	734 (74.83)	885 (95.99)	873 (97.43)	786 (98.20)	5,352 (88.63)
II	33 (5.29)	44 (3.54)	80 (6.47)	40 (3.26)	197 (4.55)	35 (2.89)	45 (3.68)	55 (5.61)	15 (1.63)	7 (0.78)	6 (0.77)	163 (2.70)
III	7 (1.06)	38 (3.10)	53 (4.32)	21 (1.72)	119 (2.75)	12 (0.96)	18 (1.50)	30 (3.06)	11 (1.19)	10 (1.12)	5 (0.64)	86 (1.43)
IV	80 (12.70)	145 (11.81)	116 (9.43)	129 (10.40)	470 (10.86)	106 (8.69)	150 (12.28)	161 (16.41)	11 (1.19)	6 (0.67)	3 (0.39)	437 (7.24)
Total (n)	628	1,230	1,230	1,239	4,327	1,220	1,220	980	922	896	800	6,038

Class I (≤ 2.10 log CFU/cm^2 or CFU/mL); Class II (results between 2.11 and 2.30 log CFU/cm^2 or CFU/mL); Class III (results between 2.31 and 2.48 log CFU/cm^2 or CFU/mL) and Class IV (> 2.49 log CFU/cm^2 or CFU/mL).
ACC – aerobic colony count; HACCP – Hazard Analysis Critical Control Points; CFU – colony forming units.
Values are expressed as number (percentages).

Table 2

Average aerobic colony count (log CFU/cm^2) determined on surfaces in school facilities for preparation and distribution of food, before and after mandatory HACCP implementation

Mandatory HACCP implementation	n	Mean values	SE
Before			
2008	627	1.42 ^{A}	0.051
2009	1,230	1.48 $^{A, a}$	0.041
2010	1,230	1.40 ^{A}	0.039
2011	1,240	1.06 $^{B, a}$	0.039
Σ	4,327	1.29 ^{b}	0.022
After			
2012	1,220	1.13 $^{A, a}$	0.030
2013	1,220	1.09 ^{A}	0.034
2014	981	1.22 $^{A, a}$	0.037
2015	922	0.64 $^{B, a}$	0.021
2016	896	0.26 $^{C, a}$	0.018
2017	800	0.75 $^{D, a}$	0.022
Σ	6,039	1.03 ^{b}	0.012

Different letters (A, B, C, D) indicate statistically significant differences ($p < 0.05$) between annual colony counts separately; different letters (a, b) indicate statistically significant differences ($p < 0.05$) only between the average aerobic colony count (Σ) and annual colony count separately.
CFU – colony forming unit; HACCP – Hazard Analysis Critical Control Points; SE – standard error.

examined period was 1.48 ± 0.041 log CFU/cm^2 in 2009, and the average ACC during all years was 1.13 ± 0.010 log CFU/cm^2. A comparison of average ACCs on surfaces in school facilities before HACCP implementation and the annual average count is shown in Table 2. There were significant differences between ACCs (log CFU/cm^2) of FCCs in school facilities that prepare and distribute food during the ten-year period. In the first trial period (2008, 2009, 2010, 2011), before the introduction of the HACCP system, the average ACCs in 2011 (1.06 ± 0.039 log CFU/cm^2) were significantly lower ($p < 0.05$) than the annual ACCs in previous years and the average ACCs for this period.

The average ACC in the first trial period before HACCP implementation (1.29 log CFU/cm^2) was significantly higher ($p < 0.05$) than the average ACC in the second period after HACCP implementation (1.03 log CFU/cm^2).

The ACCs, when calculated as annual percentages and categorized in the four classes, showed large variations in the examined years (Figure 1). The percentage of swabs with ACCs up to 2 log CFU/cm^2 increased significantly over the years. The percentage of Class I ACCs significantly increased ($R^2 = 0.493$) during the examined period (Figure 1a).

Trend analyses of the annual percentages of ACCs on surfaces from school facilities that indicated acceptable GHP from 2008 to 2017 are shown in Figures 1b and 1c. A significant percentage of surfaces was categorized as Class II ($R^2 = 0.502$) for the tested period from 2008–2018. During 2010 and 2014, more surfaces were categorized as Class II than in other examined years.

On the examined surfaces, the percentage of Class III ACCs decreased from 2008–2017. The highest annual percentages of Class III ACCs were found on surfaces in 2010 and 2014, but there was no significant decrease ($R^2 = 0.190$) of Class III ACCs during the years.

Importantly, the percentage of surfaces with ACCs above 2.48 log CFU/cm^2 (Class IV) significantly decreased ($R^2 = 0.453$) during the study period. The data obtained indicate that the application of HACCP has generally improved the microbiological status of surfaces in school facilities (Figure 1d).

The prevalence of coagulase-positive *Staphylococcus* in the swabs was highest in 2009 (1.30% of swabs were positive), but in the following years, this percentage decreased, although not significantly ($R^2 = 0.264$) (Figure 2).

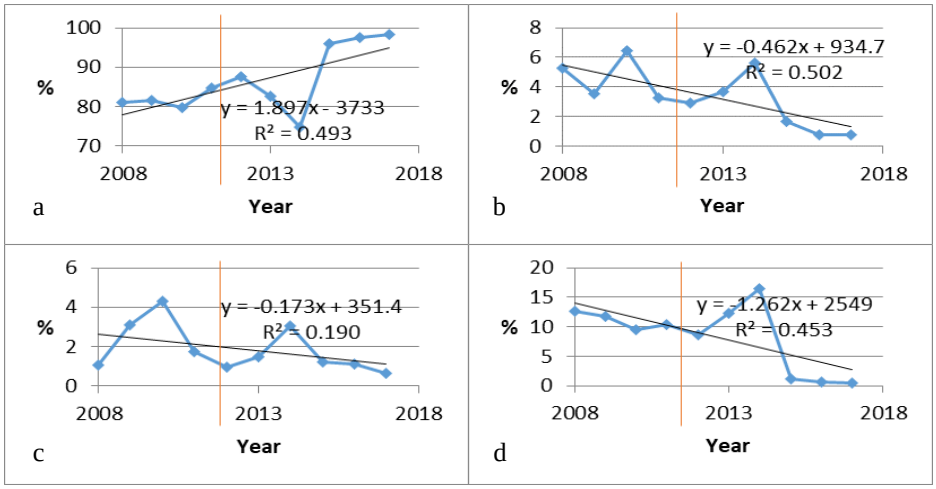

Fig. 1 – Annual percentage of four levels of aerobic colony counts isolated from surfaces in school facilities from 2008–2017.
Vertical red line represents separation of two periods.
a – Class I (up to 2.10 log CFU/cm^2); b – Class II (from 2.11 to 2.30 log CFU/cm^2); c – Class III (2.31–2.48 log CFU/cm^2); d – Class IV (over 2.48 log CFU/cm^2).
CFU – colony forming unit.

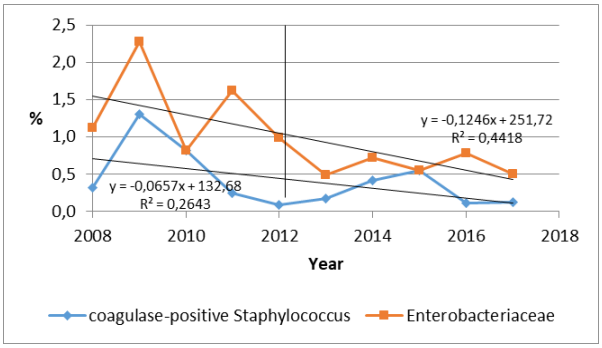

Fig. 2 – Annual percentage of surfaces in school facilities that contained coagulase-positive *Staphylococcus* and surfaces with *Enterobacteriaceae* counts above the established limit values.
The vertical black line represents the separation of two periods.

Average annual *Enterobacteriaceae* counts above the established limit values on surfaces significantly decreased ($R^2 = 0.442$) over the years and especially began to decline after the introduction of HACCP in 2012 (Figure 2).

Figure 3 shows the percentages of isolated bacteria above the established limits on three types of examined surfaces. A significantly higher ($p < 0.05$) percentage (10.53) of workers' hands harbored ACCs levels above the established limits than did work surfaces (6.21) or equipment (3.96). Moreover, a significantly lower ($p < 0.05$) percentage of sampled equipment harbored this group of bacteria than the work surfaces. A significantly higher ($p < 0.05$) percentage of swabs that harbored coagulase-positive *Staphylococcus* above the established limits were taken from workers' hands (3.60) than from equipment (0.17) or work surfaces (0.45). In addition, a significantly higher ($p < 0.05$) percentage of workers' hands (4.36) harbored *Enterobacteriaceae* above the established limits than the equipment (1.12).

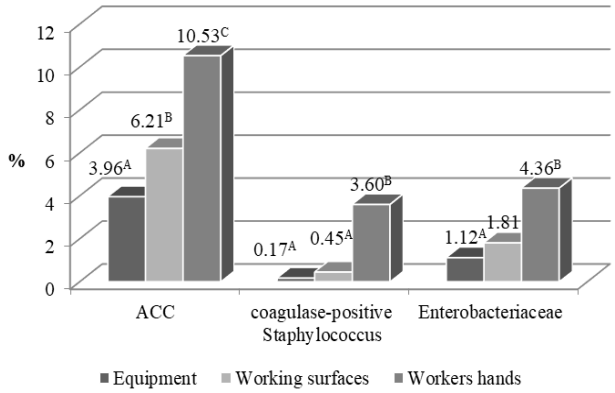

Fig. 3 – Total percentage of three surface types in school facilities that contained isolated aerobic colony count (ACC), coagulase-positive *Staphylococcus* and *Enterobacteriaceae* over the established limits during the 10-year study.
Different letters (A, B, C) indicate significant differences ($p < 0.05$) between the examined bacteria groups on the examined surfaces separately.

Discussion

Many authors defined different limits for satisfactory or unsatisfactory ACC [24–27]. In our case, ACCs were transformed from ACCs into log base 10, and all results were grouped based on the limit values of GHP. Table 1 shows the highest percentage of FCSs analyzed, throughout the entire test period, corresponding to the period when GHP was applied (Class I). Factors influencing the count of bacteria on FCSs are the season of the year, climate conditions, type of food contact material, etc. [27–30]. So far, the results are encouraging since the ACC of the largest percentage of FCSs in food preparation and distribution facilities in school institutions belonged to Class I, with low ACCs, indicating the good application of GHP. However, throughout the entire test period, the percentage of unsatisfactory ACCs was significant. That indicates that monitoring procedures, including microbial analyses, play a significant part in improving food hygiene and safety in the examined facilities [31].

Data from Table 2 indicate that general hygiene satisfied the strictest limit values (under 2 log CFU/cm^2) in all facilities. It can be concluded that the ACC levels decreased over the years, except in 2014, where the annual average ACC was significantly higher ($p < 0.05$) than the all-year average. That could be explained by the extremely high temperatures and humidity recorded in 2014. In Serbia, in the period from 1951 to 2017, 2014 was the rainiest and the hottest year in the history of temperature measurement [32]. Climate conditions have an impact on food safety, incidence, and prevalence of foodborne disease [30, 33–34]. Temperature and precipitation patterns are closely related to transport, survival, and growth of foodborne bacteria [35].

From the results shown, we can notice variations in the percentage of Class I ACCs, and variations occurred after the introduction of HACCP but generally, except for 2014, the percentage of Class I swabs increased year by year. That indicates a gradual improvement in hygiene and the appropriate application of GHP (Figure 1).

Low counts of coagulase-positive *Staphylococcus* were recorded in samples from food and work surfaces in a school catering system [36], and our data also agreed with low counts of this bacterium reported by Willis et al. [37]. A lack in temperature maintenance together with a high microbial number can lead to outbreaks of staphylococcal food poisoning [38], and could also explain some increased incidence of this pathogen in individual years of our study (Figure 2). Coagulase-positive *Staphylococcus* includes the pathogenic species *Staphylococcus aureus*, which is mostly associated with human skin and can be, thus, easily introduced into the food chain via improper food handling [39]. High levels of *Enterobacteriaceae* were ascribed by Garayoa et al. [25] to a lack of hygiene during processing. *E. coli*, a member of the family *Enterobacteriaceae*, normally lives in animal and human intestinal tracts from where it can directly spread to food, but it can also contaminate food by various other indirect pathways. Water used during food preparation and for cleaning surfaces can also be contaminated with this pathogen. The presence of *E. coli* on surfaces, such as those examined in the current study, indicates direct or indirect fecal contamination from the hands of food handlers or contaminated work surfaces or equipment [40].

Data from Figure 3 indicate the role of food handlers is truly significant in the transmission of foodborne diseases. Many authors emphasize the importance of maintaining and improving food handling practices [40–42].

Conclusion

In this ten-year study, surfaces in most school facilities had satisfactory ACCs, as measured by the surface swab technique. However, the microbiological status of workers' hands showed a need for much better practice of personal hygiene procedures. Considering all types of surfaces examined, school canteens in Novi Sad, Serbia, showed the satisfactory application of HACCP with regard

to environmental hygiene and procedures. The significant improvement in the application of GHP after the introduction of HACCP in terms of *Enterobacteriaceae* and coagulase-positive *Staphylococcus* numbers attests to appropriate control measures and adequate knowledge of food hygiene principles by the food handlers. The effectiveness of the HACCP system was confirmed, and the general average improvement of hygiene conditions in the examined canteens was also demonstrated. However, for these types of facilities, the need for enhanced supervision to support the proper implementation of good personal hygiene practices should be seriously considered.

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Knowledge and awareness of nursing students regarding human papillomaviruses infection and vaccination

Znanje i svest studenata sestrinstva o infekciji humanim papiloma virusom i vakcinaciji

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Abstract

Background/Aim. Human papillomaviruses (HPV) are the most common cause of sexually transmitted infections. Most HPV infections are transient and asymptomatic. Routine vaccination against HPV is the best prophylaxis against HPV infection. The aim of the study was to determine knowledge and risk factors about HPV infection and the attitudes towards vaccination against HPV among nursing students. **Methods.** This cross-sectional study was conducted on a random sample of 175 nursing students.The students were divided into categories according to their year of studying. Statistical data analysis was done using the *t*-test and Mann-Whitney *U* test. **Results.** Most (93.1%) respondents knew that HPV infection might cause cervical cancer and that it was a sexually transmitted infection (80.0%). Only 31.4% were aware that HPV might cause head and neck cancer, and 22.9% were aware that smoking was a risk factor for HPV infection. Male respondents ($p < 0.05$) and third-year respondents ($p < 0.05$) were significantly aware that men could also be infected with HPV. Third-year respondents were significantly aware that early sexual intercourse increased the risk of HPV infection ($p < 0.05$) and that HPV infection could be asymptomatic ($p < 0.05$). Respondents under 26 years of age ($p < 0.05$) and first-year respondents ($p < 0.05$) were significantly aware that men could as well be vaccinated against HPV. **Conclusion.** The present study found that the respondents emphasized the importance of health education by nurses and vaccination against HPV. Due to the low vaccination rate of the population, preventive measures are needed to increase public awareness about vaccination against HPV, for which nurses are also responsible.

Key words:
attitude to health; humans; knowledge; nursing care; papillomavirus; slovenia; students; vaccinaton.

Apstrakt

Uvod/Cilj. Humani papiloma virusi (HPV) su najčešći uzrok polno prenosivih infekcija. Većina infekcija HPV je prolazna i asimptomatska. Rutinska vakcinacija protiv HPV je najbolja profilaksa od ove infekcije. Cilj studije bio je da se utvrdi znanje o HPV infekciji, faktori rizika i stav prema vakcinaciji protiv HPV među studentima sestrinstva. **Metode.** Studija preseka sprovedena je na slučajnom uzorku od 175 studenata sestrinstva. Studenti su svrstani u grupe prema godini studija koju pohađaju. Za statističku obradu korišćeni su testovi *t*-test i *Mann-Whitney U* test. **Rezultati.** Većina ispitanika (93,1%) znala je da HPV infekcija može izazvati rak grlića materice i da je to seksualno prenosiva infekcija (80,0%). Samo 31,4% ispitanika znalo je da HPV može izazvati rak glave i vrata, a 22,9% ispitanika je znalo da je pušenje faktor rizika od infekcije HPV. Ispitanici muškog pola ($p < 0,05$) i ispitanici treće godine studija ($p < 0,05$) bili su značajno svesniji da i muškarci mogu biti zaraženi HPV. Ispitanici treće godine studija su imali značajno veću svest o tome da rani seksualni odnos povećava rizik od infekcije HPV ($p < 0,05$) i da infekcija HPV može biti asimptomatska ($p < 0,05$). Ispitanici mlađi od 26 godina ($p < 0,05$) i ispitanici prve godine studija ($p < 0,05$) su imali svest o tome da se i muškarci mogu vakcinisati protiv HPV. **Zaključak.** Ispitanici su naglasili važnost zdravstvene edukacije medicinskih sestara i vakcinacije protiv HPV. Zbog lošeg obuhvata stanovništva vakcinacijom potrebne su preventivne mere za podizanje svesti javnosti o vakcinaciji protiv HPV, za šta su odgovorne i medicinske sestre.

Ključne reči:
stav prema zdravlju; ljudi; znanje; nega bolesnika; slovenija; studenti; vakcinacija.

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Introduction

Papillomaviruses from the group of genotypes relevant to human medicine are called human papillomaviruses (HPV). HPV infection is the most common sexually transmitted infection [1]. The virus is transmitted via close contact with the skin or mucosa of an infected person, such as contact of the vaginal, anal, or oral area between heterosexual or homosexual partners. Routine use of condoms does not fully protect against HPV infection. HPV infection is very common after the start of sexual activity, between the ages of 20 and 24. Infection may occur during the first months after the first sexual activity. Even though the majority of sexually active adults have been exposed to HPV, new HPV infections may develop with a new sexual partner [2]. Thus, a second, milder increase of HPV infections is noticed in women around the age of 55 [3]. Risk factors for HPV infection include the early start of sexual activity (before the age of 15), numerous sexual partners (7 or more), unprotected sex, HPV oncogenicity, smoking, long-term use of oral contraceptives (progesterone), other sexually transmitted infections and diseases [herpes simplex, chlamydia, gonorrhea, human immunodeficiency virus (HIV) infections], low socioeconomic status, etc. [4, 5].

Most HPV infections are transient and asymptomatic and do not cause any clinical problems [6]. Due to cell-mediated immunity, infection is usually transient and mostly (80–90%) resolves by itself within one to two years. Since a person is often unaware of their infection, HPV can be rapidly transmitted between sexual partners [7, 8]. Rarely do oncogenic HPV types remain present (10%), causing persistent infection. The persistence of the infection is associated with oncogenesis [5]. After several years (7–10 years), persistent HPV infection may progress to precancerous lesions or cervical cancer and other anogenital and/or oropharyngeal cancers [4, 9].

Routine vaccination against HPV is the best prophylaxis against HPV infection and HPV-associated diseases, including some cancers; therefore, it was introduced into national immunization programs in all EU countries, except Poland, between 2006 and 2018 [10]. The primary target groups are youths between 9 and 14 years old previously not exposed to HPV infection or those who have not been sexually active. This is due to the fact that the vaccine is more immunogenic in people younger than 26 years. Furthermore, vaccination against HPV is most effective before potential exposure to HPV infection, i.e., before the first sexual contact. Protection following vaccination against HPV lasts for at least 10 years and is expected to be long-lasting [11, 12]. There are currently three licensed prophylactic vaccines against HPV. The vaccination scheme for all three vaccines depends on the age of the recipient. For those below 15 years, two doses are recommended (at 0, 6 months), and three doses (at 0, 2, 6 months) for those above 15 years [13, 14]. Vaccination is also effective after the start of sexual activity, but the person is only protected against those virus genotypes with which they have not been infected at the time of vaccination [15]. In 2015, the Advisory Committee on Immunization Practices introduced a catch-up vaccination for young women (between 13 and 26 years) and young men (between 13 and 21 years, and 26 years for high-risk men) who have not previously been vaccinated [6]. Catch-up immunization programs have been established in 10 countries [16]. Anti-HPV antibodies persist several years after vaccination at levels significantly higher than those following the natural course of infection [14]. Vaccination efficacy is high, preventing up to 90% of cervical cancer cases. In addition to girls, some countries around the world also vaccinate boys. This option has been available in Denmark since 2006. Until 2014, only 4,239 Danish males between 9 and 26 years were vaccinated [17]. Since 2013, Australia has provided free vaccination for boys up to the age of 15 [18]. During HPV infection, seroconversion in men is lower, so vaccination against HPV is the most reliable method for immunoprotection against HPV infection and the risk of cancer. The proportion of HPV infections in young men is equal to that in young women; however, the proportion of infections in women considerably declines with age, which is not the case seen in older men [19].

In Slovenia, a 2-valent vaccine has been available since 2007, 4-valent since 2006, and 9-valent since 2016. Girls are vaccinated routinely in the 6th grade of primary school. The vaccination rate of girls with the second dose against HPV increased by 10% (from 49.5% in 2017/2018 to 59.3% in 2018/2019). Vaccination for boys is covered by self-payment, but an extension of the Vaccination Program, including vaccination against HPV for boys, has been proposed [20]. In 2017, coverage with ≥ 1 dose of vaccine against HPV in the USA was 65.5% in adults between the ages of 13 and 17 [21].

Due to the low vaccination rate of the population, preventive measures to increase public awareness about vaccination against HPV are needed. Healthcare professionals need evidence-based expertise since their task is to make efforts to reduce the number of HPV infected people [22] through health education and preventive programs [23]. In doing this, they should be focused on the younger population, which is particularly at risk, also due to poor awareness [24]. The aim of the study was to determine knowledge and risk factors about HPV infection and the attitudes towards vaccination against HPV among nursing students.

Methods

All 214 nursing students who attended the 3-year undergraduate nursing program at the Faculty of Health Sciences in Slovenia were invited to participate in the research. This cross-sectional study included 175 nursing students in total who completed the questionnaire. The response rate was 82%. First and second-year nursing students did not have theoretical and clinical education about women's health nursing. Third-year nursing students received women's health nursing theoretical content (45 hrs). The study was conducted from December 2017 to January 2018.

This study was reviewed and approved by the institutional review board at the Faculty of Health Sciences, University of Ljubljana. A written explanation of the study's

procedure was given to the participants. The autonomy of participating and the right to stop and withdraw from the study at any time were also explained. The questionnaires were distributed during lecture hours. The content of the study was explained to the respondents. The questionnaires were returned directly to the researchers in a closed envelope. Instructions on how to complete the survey were included as well as a cover letter indicating the study's purpose. The nursing students participated voluntarily and were reassured of data confidentiality, as well as anonymity.

The questionnaire was constructed on the basis of the literature review [25–29]. A questionnaire was specially designed for the purposes of this study. A pilot study was conducted among 10 first-year nursing students. The reliability of the questionnaire, according to Cronbach's alpha, was 0.74. The questionnaire contained questions concerning demographic data (age, gender, place of residence, year of study) as well as 15 claims of risk factors for HPV infection, 9 claims regarding vaccination against HPV, and 9 attitudes on preventive awareness-raising about infection and vaccination against HPV in Slovenia.

Descriptive statistics were derived and expressed as frequency and percent. Categorical data were compared using the *t*-test for independent samples for between-group differences by gender and the Mann-Whitney *U* test for between-group differences by age, year of study, and place of residence. A *p*-value < 0.05 was considered statistically significant. Data were analyzed by SPSS IBM v. 23 for Windows (IBM Corporation).

Results

The sample included 175 nursing students. The median age among all respondents was 20 (range 19–43 years), and the mean age was 21.5 ± 4.7 years. The majority of the sample was female (78.9 %) who lived in the rural area (60%) and were in their first year of study (40.6 %) (Table 1).

Most respondents (93.1%) knew that HPV infection might cause cervical cancer, that it was a sexually transmitted infection (80.0%), that oral contraceptives did not protect against HPV infection (90.9%), and that HPV infection could not be cured with antibiotics (81.7%). Only 31.4% were aware that HPV might cause head and neck cancer, and only 22.9% were aware that smoking was a risk factor for HPV infection. Majority (58.3%) of respondents knew that men could be infected with HPV; there were statistically significant differences ($t = 2.649$; $p < 0.05$) between the male and female respondents and between the third-year respondents and respondents of other years of the study ($Z = -1.987$, $p < 0.05$). Similarly, regarding awareness that HPV is transmitted through vaginal, oral, and anal sex there were statistically significant differences ($t = 2.070$; $p < 0.05$) between the male and female respondents, female and between the third-year respondents and respondents of other years of the study ($Z = -1.875$, $p < 0.05$). Regarding awareness that sexual intercourse increases the risk of HPV infection, that HPV infection can be asymptomatic, and that it can cause genital warts, there were statistically significant differences between respondents who were in the third year and those who were in other years of the study ($Z = -1.728$, $p < 0.05$; $Z = -2.720$, $p < 0.05$; $Z = -3.021$, $p < 0.05$; respectively). That HPV infection does not cause herpes, there was a statistically significant difference ($Z = -2.288$; $p < 0.05$) between respondents over 26 years and respondents below 26 years of age (Table 2).

Most respondents knew that vaccination against HPV in Slovenia was optional (84.6%), that a person should get vaccinated even if they had only one sexual partner (82.9%), and that vaccination should be performed before the first sexual intercourse (77.1%). They were less aware that men could also be vaccinated (52.6%) and that vaccination prevented genital warts (46.3%). The men can also be vaccinated against HPV; there were statistically significant differences between the respondents below 26 years and those > 26 years ($Z = -2.635$; $p < 0.05$) and between the first-year respondents and those in other years of the study ($Z = -2.359$; $p < 0.05$). Vaccination against HPV in Slovenia is not mandatory; there was a

Table 1

Socio-demographic characteristics of the study participants

Parameters	Participants n (%)
Age (years)	
19–26	159 (90.9)
> 26	16 (9.1)
Gender	
male	37 (21.1)
female	138 (78.9)
Place of residence	
rural	105 (60.0)
urban	70 (40.0)
Year of study	
first	71 (40.6)
second	63 (36.0)
third	41 (23.4)

statistically significant difference (Z = -2.144; $p < 0.05$) between respondents who were in the third year and those in other years of the study. Furthermore, vaccination against HPV can be performed after the first sexual intercourse; there was a statistically significant difference (Z = -1.987; $p < 0.05$) between respondents who were below 26 and those over 26 years of age (Table 3).

Table 4 shows that respondents believe there is insufficient health education focused on preventive protection against HPV infection. Only 44% of respondents received information on protection against HPV infection at systematic health check-ups, and only 22.9% had heard of vaccination against HPV in the media. Regarding knowledge that healthcare personnel do not raise sufficient awareness among the youth regarding the risk factors for HPV infection, there was a statistically significant difference ($t = 2.620$; $p < 0.05$) between male and female respondents who were in the third year and those who were in other years of the study. Nurses emphasized the importance of vaccination against HPV; there was a statistically significant difference (Z = -1.963; $p < 0.05$) between first-year respondents and those in other years of the study. Those living in rural areas agreed that they are insufficiently aware of HPV infection (Z = -2.044; $p < 0.05$), and those living in urban areas agreed that they do not know enough about vaccination against HPV (Z = -2.045; $p < 0.05$). Regarding the attitude that the third-year respondents believe that vaccination against HPV infection is effective and would thus recommend it, there was a statistically significant difference (Z = -2.658; $p < 0.05$) between respondents in the third year and those who were in other years of the study.

Table 2

Knowledge regarding risk factors and HPV infection

Knowledge claims	Correct answer n (%)	*p*-value
HPV infection can cause cervical cancer (T)	163 (93.1)	ns
HPV infection is sexually transmitted (T)	140 (80.0)	ns
Men can also be infected with HPV (T)	102 (58.3)	0010* 0.047‡
HPV infection can be cured with antibiotics (F)	143 (81.7)	ns
HPV infection can be asymptomatic (T)	130 (74.3)	0.007‡
HPV infection can cause herpes (F)	79 (45.1)	0.022‡
HPV is a strong risk factor for head and neck cancer (T)	55 (31.4)	ns
HPV infection can cause genital warts (T)	121 (69.1)	0.003‡
HPV is transmitted via blood and saliva (F)	98 (56.0)	ns
Contraception pills can protect against HPV infection (F)	159 (90.9)	ns
We cannot get infected if we have a single sexual partner (F)	130 (74.3)	ns
Early sexual intercourse can increase the likelihood of HPV infection (T)	100 (57.1)	0.050‡
HPV infection can be transmitted through vaginal, oral, and anal sex (T)	138 (78.9)	0.042* 0.048‡
Condom protects against HPV infection, but not 100% (T)	135 (77.1)	ns
Smoking can be a strong risk factor for HPV infection (T)	40 (22.9)	ns

HPV – human papillomavirus; T – true; F – false; *Gender difference – $p < 0.05$, statistical significance determined by the *t*-test; †Age difference – $p < 0.05$, statistical significance determined by the Mann-Whitney *U* test; ‡Year of study difference – $p < 0.05$, statistical significance determined by the Mann-Whitney *U* test.

Table 3

Knowledge regarding vaccination against HPV

Knowledge claims	Correct answer n (%)	*p*-value
Vaccination against HPV in Slovenia is optional (T)	148 (84.6)	0.032‡
Vaccination cannot protect us against all HPV genotypes (T)	115 (65.7)	ns
Men can also be vaccinated against HPV (T)	92 (52.6)	0.008‡ 0.018‡
Vaccination against HPV can prevent the development of genital warts (T)	81 (46.3)	ns
There are several types of vaccines for different HPV genotypes (T)	99 (56.6)	ns
Vaccination against HPV is recommended before the first sexual intercourse (T)	135 (77.1)	ns
Women and men can be vaccinated against HPV even after the first sexual intercourse until the age of 26 (T)	102 (58.3)	0.047‡
There is no need to be vaccinated against HPV if a person has only one sexual partner (F)	145 (82.9)	ns
A person vaccinated against HPV can still develop various cancers caused by HPV (T)	130 (74.3)	ns

HPV – human papillomavirus; T – true; F – false; ‡Year of study difference – $p < 0.05$, statistical significance determined by the Mann-Whitney *U* test.

Table 4

Attitudes on preventive awareness-raising about infection and vaccination against HPV in Slovenia

Awareness-raising attitudes	Correct answer n (%)	*p*-value
Healthcare personnel do not raise sufficient awareness among the young population regarding risk factors for HPV infection	156 (89.1)	
There is insufficient health education focused on preventive protection against HPV infection	160 (91.4)	0.010*
I am insufficiently aware of HPV infection	50 (28.6)	ns
I was instructed about protection against HPV infection during systematic health check-ups	77 (44.0)	0.041§
Nurses should place more emphasis on the importance of vaccination against HPV	149 (85.1)	ns
Nurses encourage the young to be vaccinated against HPV	103 (58.9)	ns
I believe I do not know enough about the vaccine and vaccination against HPV	142 (81.1)	0.050‡
I have heard a lot about vaccination against HPV in the media	40 (22.9)	0.041§
I believe that the vaccine against HPV is effective and would thus recommend vaccination	114 (65.1)	ns

HPV – human papillomavirus; *Gender difference – $p < 0.05$, statistical significance determined by the *t*-test; ‡Year of study difference – $p < 0.05$, statistical significance determined by the Mann-Whitney *U* test; §Place of residence difference – $p < 0.05$, statistical significance determined by the Mann-Whitney *U* test.

Discussion

Our study showed there is insufficient knowledge regarding HPV infection among nursing students. Most respondents (80%) knew that HPV infection was sexually transmitted, that it could cause cervical cancer (93.1%) and that HPV infection could be protected with condom use (77.1%). Among Italian students, 84.7% knew that HPV infection could cause cervical cancer [28], 54.1% of Pakistani students [27], and 90.4% of nurses in the UK [29]. In Turkey, 88.7% of female students did not know how HPV infection was transmitted, as much as 90.9% of female students were not aware of what constituted adequate protection against such infection, and only 8.7% were aware of the vaccine against HPV [30]. In our study, 77.1% of respondents knew that condoms did not provide complete protection against HPV infection in comparison with 35.2% among male USA students [25]. Only 56% of respondents in our study knew that HPV genotypes were not found in urine, blood, or saliva, and only 45.1% knew that HPV did not cause herpes. Lower awareness was found among English women (19–26 years); only 10% were aware that HPV was not transmitted through the exchange of body fluids, and 13% that it did not cause herpes [16]. As many as 62.2% of USA students believed that HPV could cause herpes [26]. Our respondents knew more about HPV infection, its consequences, and effective protection against HPV because they received general information several times during adolescence and education. However, our respondents knew too little about the link between HPV infection and head cancer and smoking.

In our study, it was found that only 58.3% of respondents knew that men could also get infected with HPV. It is positive that male respondents were statistically significantly aware that men could also get infected with HPV and that infection was transmitted via sexual intercourse. Among Italian nursing students, 85.6% knew that men and women could develop disease following HPV infection [28]. Among Pakistani students, 55% knew that men and women could get infected with HPV [27]. By contrast, American Indian graduate male students were aware of their insufficient knowledge about HPV; unlike women, they believed they were not endangered by HPV infection. Most men said that women were the ones who should be aware of the risk of HPV infection, while women believed that they were solely responsible for the prevention of HPV infection, not men [31]. Similarly, among Hungarian men aged 18 and above, as many as 82.8% believed that HPV infection was irrelevant for them [32]. Similar poor awareness was observed in young Moroccan women (18–30 years); only 45% agreed that men could get infected with HPV [33]. In the USA, it was found that among men and women aged between 14 and 69, the prevalence of HPV infection was higher in men [34]. Male respondents knew that they too could become infected with HPV, which could be explained by the more successful promotion of vaccination against HPV for boys as well.

Our study showed that only 31.4% of respondents knew that HPV infection was a risk factor for developing head and neck cancer, and only 22.9% knew that smoking was a risk factor for HPV infection. Among men in the USA, it was found that only 31.5% knew that HPV in men could cause oropharyngeal cancer [35]. The lack of knowledge about the link between HPV infection and head cancer among respondents suggests the need to place greater emphasis on these links during education.

Most respondents (77.1%) from our study were aware that vaccination against HPV was recommended before the first sexual intercourse. Only 52.6% of respondents knew that men could be vaccinated against HPV; male respondents under the age of 26 were statistically significantly more aware of this. Only 58.3% of respondents knew that both men and women could get vaccinated until the age of 26 and also after the first sexual intercourse; third-year respondents were statistically significantly aware of this. Among New Mexico nursing students, it was found that more than half were unaware that men could get infected with HPV and be vaccinated against it [26]. In the USA, it was found that only 50% of men had heard of HPV, of which 53% were unaware that they could get vaccinated against HPV [36]. Insufficient

knowledge was also observed in India among medical students, of whom only 18.8% knew that men and women could be vaccinated up to the age of 26 [24]. As many as 65% of Swedish girls with a mean age of 18 years were vaccinated against HPV after their first sexual intercourse [37]. Since there are no screening tests for men showing the presence of HPV, they are unaware of their infection; it is detected only after the development of a malignant neoplasm. Therefore, their best protection, in addition to safe sex, is vaccination. However, very few men are aware that, during the last decade, they have also had the possibility of being vaccinated against HPV [38]. An important finding of our study was the fact that male respondents, both under 26 years of age and third-year respondents, knew about vaccination against HPV, which could be explained by better preventive action for young people. However, this result could be related to the direction of the nursing study. Thus, some municipalities in Slovenia already allow free vaccinations for boys.

Our study showed the following: 65.7% of respondents knew that the vaccine against HPV did not protect against all genotypes; only 65.1% would recommend vaccination against HPV; only 44% were instructed about protection against HPV infection at systematic healthcare check-ups; 85.1% believed that nurses should emphasize the importance of vaccination against HPV; 89.1% of respondents believed that healthcare personnel did not do enough to raise awareness of the risk factors for HPV infection among youth. 68% of South Carolina students received information on HPV from healthcare professionals [39]. Healthcare personnel can significantly contribute to the decision on vaccination. In the UK, almost all nurses (98.9%) recommend vaccination against HPV, and most of them (88%) believe that vaccines should be offered to boys as well [29]. In the USA, too, healthcare personnel encouraged 83% of homosexual persons (aged 18–26) to get vaccinated against HPV [40]. Healthcare professionals should provide effective healthcare education in order to increase awareness and reduce the burden of precancerous changes, which have become increasingly common in the last decade [41]. In particular, they should encourage the youth to get vaccinated and inform them about the consequences of (too) early, high-risk, and unprotected sex [26]. Collective immunity can only be achieved through the vaccination of girls and boys against HPV. One would expect that nursing students, being future healthcare professionals, would trust the efficacy of the vaccine.

This study has the limitation of being a single-center study with a small sample size. The survey was conducted in only one geographical area. Thus, the results cannot be generalized to all nursing students. Another important limitation was that we surveyed nursing students of all academic years, with only third-year students completing lectures in oncology and gynecology. The important aspect of this study was to demonstrate to the respondents the emphasized importance of health education for nurses.

Conclusion

The present study found there was insufficient knowledge among nursing students regarding risk factors and vaccination against HPV infection. The respondents emphasized the important role of nurses in raising awareness about HPV. Being future healthcare professionals, they need evidence-based expertise about HPV infection and its consequences and vaccination against HPV, allowing them to act preventively. Improving the knowledge regarding HPV and raising awareness among young girls and boys provides great health benefits by reducing the morbidity and mortality associated with HPV infection and transmission. Similarly, young people need to trust care professionals whose expertise contributes to increased awareness, encouraging them to get vaccinated against HPV.

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Habits, attitudes, and behavior of refugees and migrants in Serbia concerning oral health

Navike, stavovi i ponašanje izbeglica i migranata u Srbiji u vezi sa oralnim zdravljem

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Abstract

Background/Aim. A large number of refugees and migrants have passed through the Republic of Serbia in recent years. Oral health is one of the key indicators of general health. The aim of this study was to investigate self-reported oral health, oral health related habits, nutrition, and the use of dental services among refugees and migrants in Serbia. **Methods.** A total of 226 migrants participated in the study. Participants were accommodated in the migrant centers Obrenovac and Krnjača in Belgrade, Serbia. All participants were given a questionnaire in order to examine oral health habits, attitudes, and behavior among refugees and migrants. The questionnaire consisted of 29 questions. SPSS 24 statistical software was used to analyze answers from the questionnaire. **Results.** Out of 226 examinees, 40 were female, and 186 were male. The majority (87.6%) were adults, and 12.4% were children. The results showed that refugees and migrants who spent 200–300 € per month consumed alcohol (33.3%) and tobacco (61.1%) the most. Only 10.8% of men answered they had been to the dentist in Serbia, whereas 35% of women had the same answer ($p = 0.000$). The research also showed that most women (67.5%) brush their teeth 2-3 times a day, and noticeably fewer men (37.1%) had the same habit. Fluoride supplements were used by 78.7% of examinees. Among the most common reasons for the last visit to the dentist were pain (36.9%) and regular checkups (22.5%). **Conclusion.** Preservation of oral health of refugees and migrants in Serbia depends on various factors. Improving and preserving the good general and oral health of refugees and migrants should be a public healthcare priority.

Key words:
attitude to health; habits; oral health; preventive dentistry; refugees; risk factors; serbia; transients and migrants.

Apstrakt

Uvod/Cilj. Veliki broj izbeglica i migranata je poslednjih nekoliko godina prošao kroz Srbiju. Oralno zdravlje je jedan od ključih pokazatelja opšteg zdravlja. Cilj istraživanja bio je da se sprovede samoprocena oralnog zdravlja i utvrde navike vezane za oralno zdravlje, ishranu i korišćenje stomatoloških usluga izbeglica i migranata u Srbiji. **Metode.** U istraživanju je učestvovalo ukupno 226 ispitanika. Učesnici su bili smešteni u migrantskim centrima Obrenovac i Krnjača u Beogradu, u Srbiji. Svim ispitanicima je data anketa sa ciljem da se istraže navike, stavovi i ponašanje u vezi sa oralnim zdravljem izbeglica i migranata. Upitnik se sastojao od 29 pitanja. Statistički softver SPSS 24 korišćen je za analiziranje odgovora dobijenih u anketi. **Rezultati.** Od ukupno 226 ispitanika bilo je 40 ženskog, a 186 muškog pola. Većina ispitanika bila je punoletna (87.6%), a 12.4% su bila deca. Rezultati pokazuju da su izbeglice i migranti koji troše 200–300 € mesečno najviše konzumirali alkohol (33.3%) i duvan (61.5%). Svega 10.8% muškaraca je odgovorilo da je bilo kod stomatologa u Srbiji, dok je 35% žena dalo isti odgovor ($p = 0.000$). Istraživanje je, takođe, pokazalo da je većina žena (67.5%) prala zube 2–3 puta dnevno, dok znatno manje muškaraca ima tu naviku (37.1%). Suplemente fluorida koristilo je 78.8% ispitanika. Kao neki od najčešćih razloga za posetu stomatologu navedeni su bol (36.9%) i redovna kontrola (22.5%). **Zaključak.** Očuvanje oralnog zdravlja izbeglica i migranata u Srbiji zavisi od različitih faktora. Unapređivanje i očuvanje dobrog opšteg i oralnog zdravlja izbeglica i migranata trebalo bi da budu prioriteti javnog zdravlja.

Ključne reči:
stav prema zdravlju; navike; oralno zdravlje; stomatologija, preventivna; izbeglice; faktori rizika; srbija; prolaznici i migranti.

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Introduction

Since the peak of the migration crisis in 2015, like many European countries, Serbia was struck with hundreds of thousands of refugees and migrants traveling across European borders [1].

The United Nations High Commissioner for Refugees (UNHCR) stated that there were 30,216 newly arrived asylum seekers and migrants in Serbia in 2019. In the same year, the number of arrivals of unaccompanied/separated children was almost twice as high as the year before, with a total of 3,777 children. The majority of the asylum seekers and migrants were men (76%) and much fewer women (18%) and children (6%). When talking about nationalities, 51% came from Afghanistan, 13% from Syria, 7% from Pakistan, 6% from Bangladesh, 6% from Iran, 6% from Iraq, and 11% from other countries [2].

The effects of the migratory process can be noticed as changes in social determinants of health, lack of access to healthcare, interrupted care, poor living conditions, or others. That means general health could be in jeopardy. Healthcare systems and healthcare providers are the ones put to the test when this happens.

Most common regional health policies recommend that emergency and urgent care be available to all refugees and migrants, regardless of their legal status [3]. The Law on Healthcare of Serbia, specifically article 236 of this document, states that a person without citizenship, refugees, people seeking asylum, or registered foreigners seeking asylum, have the right to healthcare [4].

Knowing that oral health is one of the key indicators of general health, well-being, and quality of life, we should thoroughly examine the impact of oral health habits in order to preserve and improve oral health among migrants and refugees [5]. The aim of this study was to provide information about oral health habits, attitudes, and behavior towards oral health and indicate how public healthcare can contribute to oral health preservation and improvement among refugees and migrants in Serbia.

Methods

This study was approved by the Commissariat for Refugees and Migration and the Ministry of Health of the Republic of Serbia. Approval from the University of Belgrade Faculty of Dental Medicine's Research Ethics Committee was obtained prior to this study (registration number: 36/16). The research took place in refugee centers in Obrenovac and Krnjača in Serbia from November through December 2019.

Participants

A total of 226 refugees and migrants took part in this exploratory cross-sectional study. Inclusion criteria for the respondents in the study were a signed consent form and the ability to understand and answer the questions independently or with the help of a qualified translator. Exclusion criteria were refusal to take part in the study and refusal of the parent or guardian to have their child participate in the study. Participation in this study was anonymous and voluntary. All participants were fully informed before giving consent or allowing their children to take part in the research. Parents answered questions on behalf of their children.

Data collection

The survey was carried out in migrant centers in Obrenovac and Krnjača. All questions were asked orally to ensure equality. Parents answered questions for children involved in the study. One certified dentist (the first author) conducted the interviews and one dental student (the second author) noted and saved the data. The interviews were carried out in Serbian, English, Pashto, and Farsi language with the help of certified translators in the migrant centers. Each interview took approximately 20 min.

The survey included both closed and open-ended questions. Participants were asked if they had any bad oral health habits (teeth grinding/thumb sucking/mouth breathing/chewing on one side of the mouth/no bad habits) and if they consumed tobacco (yes/no) or alcohol (yes/no).

Furthermore, we asked participants about their food regimen, such as the frequency of consuming sweetened drinks/juices, sweets, and fruits (daily/several times per week/several times per month/rarely/never).

Questions about the frequency of tooth brushing (never/2–3 times per month/once per day/two or more times per day), the use of fluoride supplements (toothpaste/mouthwash/fluoride tablets/fluoride varnish/tooth gel/no fluoride supplements), and oral hygiene utensils (toothbrush/interdental brush/oral irrigator/toothpick/dental floss/no utensils) were asked in order to investigate oral hygiene habits of migrants and refugees.

Oral health problems (yes/no/I do not know) and the number of dental visits (once/twice/three or more times/ I have not been to the dentist in the last year) in the year prior to the research were questions of great value for this research. We also inquired about the reason for the last visit to the dentist (pain/trauma/swelling/gum bleeding/regular checkup/none of the above). Questions about the satisfaction of overall and oral health (very dissatisfied/dissatisfied/neither satisfied nor dissatisfied/satisfied/very satisfied) were also incorporated in this study.

A specific part of this research was the attitude of migrants and refugees toward dental healthcare in Serbia. We inquired whether the participants knew they were provided free dental care in Serbia (yes/no/I do not know), as well as their thoughts on whether there was a language barrier that would endanger their dental treatment (yes/no). Participants were asked if they had been to a dentist in Serbia (yes/no) or had ever been denied dental treatment (yes/no). Those who had dental treatment in Serbia were asked if they were satisfied with the service (very dissatisfied/dissatisfied/neither satisfied nor dissatisfied/satisfied/very satisfied).

The participants were also asked about their age, gender (male/female), marital status (single/married/divorced/widowed), country of origin, time spent in Serbia (under 10

days/10–30 days/1–2 months/more than 2 months), migrant and refugee center they were situated at (Obrenovac/Krnjača), and the amount of money at their disposal per month (less than 100 €/100–200 €/200–300 €/more than 300 €). In addition to these questions, participants answered questions about their own, their mother's, and their father's education level (primary school or less/high school/bachelor's degree/master's degree/PhD/unknown).

Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, version 24.0; Armonk, NY: IBM Corp.) The Chi-squared test was used to determine the significance of differences between two independent groups. In the process of examining the relationship between variables, the ANOVA test was used. The level of significance was set at 5% ($p < 0.05$).

Results

Socio-demographic characteristics

This study included 226 refugees and migrants staying in migrant centers in Serbia. Single male refugees and migrants were situated in Obrenovac, whereas families, children, and female refugees and migrants were in Krnjača. Their average age was 23 ± 8.24, aged 1 to 64. The ages of children that participated in the study were from 1 to 17, with an average age of 10 ± 3.87, whereas the youngest adult was 18 and the eldest 64. The demographic and socio-economic characteristics of the examinees are shown in Table 1. The results showed that refugees and migrants originate from Asian and African countries: Afghanistan 73.89%, Iran 7.52%, Pakistan 4.87%, Syria 4.42%, Iraq 3.54%, Somalia 3.1%, Yemen 0.88%, Cameroon 0.44%, Ethiopia 0.44%, Lebanon 0.44%, and Sudan 0.44%. Concerning educational attainment, participants answered in the majority that their mother finished only primary school or less (41.6%). Similar results were obtained when asked about their father, with 38.1% of the participants answering primary school or less and 23.9% high school. As for the participants themselves, 35.8% finished primary school or less, 35.4% finished high school, and 25% did not answer the question.

Habits

When answering the question about bad habits, most participants (46.5%) answered they did not have any of the following: unilateral chewing, teeth grinding, thumb sucking, or mouth breathing. A much smaller percentage had bad habits: 22.9% unilateral chewing and 19.8% teeth grinding. A large percentage of refugees and migrants consume tobacco (48%). Even though 86.7% of participants do not drink alcohol, refugees and migrants who have 200–300 € per month at their disposal consume alcohol the most, as well as tobacco (61.1%).

Table 1

Demographic and socio-economic characteristics of examinees

Parameter	Participants	
	male (n = 186)	female (n = 40)
	n (%)	n (%)
Migrant center		
Obrenovac	152 (81.7)	0 (0)
Krnjača	34 (18.3)	40 (100)
Age (years)		
< 18	13 (7)	15 (37.5)
≥ 18	173 (93)	25 (62.5)
Months lived in Serbia		
< 1	42 (22.6)	15 (37.5)
1–2	46 (24.7)	7 (17.5)
> 2	94 (50.5)	17 (42.5)
no answer	4 (2.2)	1 (2.5)
Marital status		
Single	127 (68.3)	19 (47.5)
Married	54 (29)	19 (47.5)
Divorced	1 (0.5)	0 (0)
Widowed	2 (1.1)	1 (2.5)
no answer	2 (1.1)	1 (2.5)
Money on disposal per month (in euros)		
< 100	118 (63.4)	34 (85)
100–200	27 (14.5)	3 (7.5)
200–300	18 (9.7)	0 (0)
> 300	14 (7.5)	0 (0)
no answer	9 (4.8)	3 (7.5)

Food regimen

Food is provided in both migrant centers, Krnjača and Obrenovac. In addition to the provided food, refugees and migrants can buy and consume other foods. The majority of respondents ate sweets on a daily basis or a few times a week (Table 2). The male population drinks sweetened juices more often than the female ($p = 0.008$). Statistical analysis showed that the duration of the stay of refugees and migrants in Serbia affected the consumption of fruits ($p = 0.008$). According to the results, 50% of migrants who stayed in Serbia for a longer period of time, longer than one month, ate fruits daily, and 28.4% of them ate fruits a few times a week.

Oral hygiene

Good oral hygiene is mandatory for good oral health. The results show that 42.5% of participants brush their teeth two or more times a day, and 19% of refugees and migrants mentioned they brush their teeth once a day. There was statistical significance between men and women concerning the frequency of brushing teeth (Figure 1). The results show a statistical significance of $p = 0.000$ in the correlation between the frequency of tooth brushing and satisfaction with oral health. A noticeably large percentage of participants used fluoride supplements (79%). The most preferred fluoride supplement among migrants and refugees in Serbia is toothpaste.

Dental healthcare

The majority of respondents ranked their general health as satisfactory (64.6%). Twice as many men answered that their oral health is dissatisfactory (22%), whereas 10% of women had the same answer ($p = 0.002$). Out of the total number of participants, 36.7% of refugees and migrants mentioned having had problems with oral health in the past year. Although there is a widespread need for dental treatment, 57.1% of the participants have not been to the dentist in the past year. The most common reason for their last visit to the dentist was pain (39.2%). Refugees and migrants who have between 200 € and 300 € per month at their disposal had the highest percent (50%) of last visits to the dentist caused by pain.

Dental healthcare in Serbia

The majority of the participants answered that they did not know if dental treatment in Serbia was free (45.5%); the rest were divided, 27.5% stating it was free and 27% stating it was not. Most of the single participants (60%) believed no language barrier would endanger their dental treatment. However, married participants were not as sure, with a total of 47.9% answering there was a language barrier. The results show that women have been to a dentist in Serbia more often than men (Table 3). Based on the results obtained, a total of 38 refugees and migrants were in a situation where a dentist

Table 2

Consumption of sweets, fruits, and sweetened drinks/juices among refugees and migrants (%)

Parameter	Daily	Several times per week	Several times per month	Rarely	Never	No answer
Consumption of sweets	35	30.5	7.1	18.6	8.4	0.4
Consumption of fruits	45.5	29.2	6.2	12.4	5.8	0.9
Consumption of sweetened drinks/juices	36.7	28.8	7.1	18.6	10.6	0.9

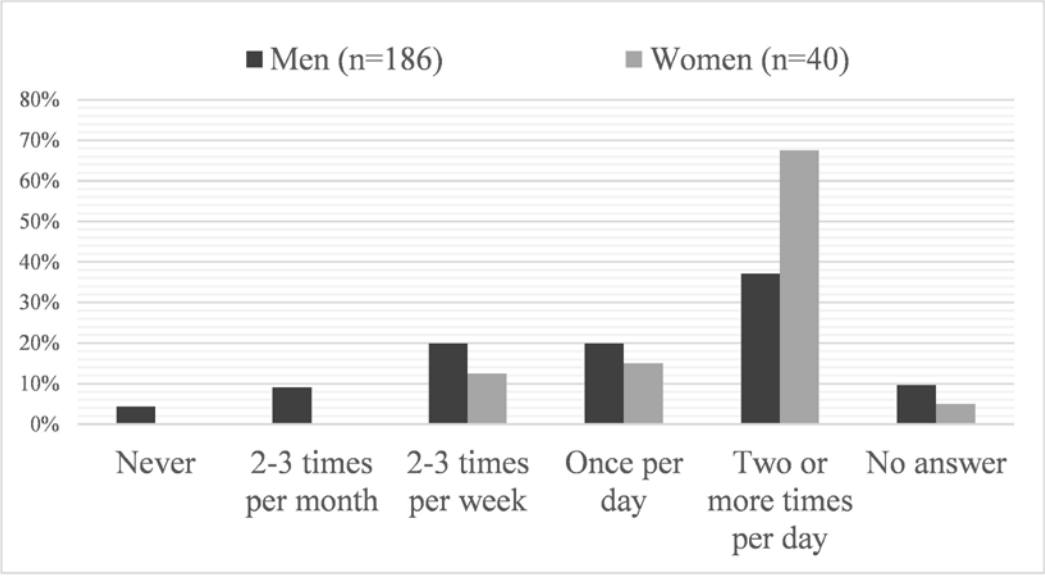

Fig. 1 – Frequency of tooth brushing among refugees and migrants (%).

Table 3

Dental healthcare in Serbia for migrants and refugees

Parameter	Participants male (n = 186)	Participants female (n = 40)	*p*-values*
	n (%)	n (%)	
Language barrier endangering dental treatment			0.16
yes	65 (34.9)	14 (35)	
no	98 (52.7)	25 (62.5)	
no answer	23 (12.4)	1 (2.5)	
Visit to a dental office in Serbia			0.00
yes	20 (10.7)	14 (35)	
no	159 (85.5)	26 (65)	
no answer	7 (3.8)	0 (0)	
Been refused dental treatment in Serbia?			0.17
yes	35 (18.8)	3 (7.5)	
no	143 (76.9)	34 (85)	
no answer	8 (4.3)	3 (7.5)	

***Pearson's χ^2 test.**

refused to give them dental treatment. Most participants (44.1%) were satisfied with dental treatment in Serbia.

Discussion

In 2019, 14 reception centers and 6 centers for asylum were at the disposal of refugees and migrants across Serbia. The total capacity of all available migrant centers is approximately 5,890 [6]. European Union Agency for Fundamental Rights (FRA) report from May 2017 states that the majority of refugees and migrants in European Union (EU) countries have only a primary school degree [7]. UNHCR statistics show that 91% of the world's children attend primary school, whereas 61% of refugee children have the same chance. Serbia follows other European countries and provides children with primary school education [8]. When comparing the monthly budget of refugees and migrants, we should take into consideration that in Serbia, in 2019, the minimum wage was approximately 30,000 RSD (≈ 255 €), and the basic amount of monthly social assistance was 8,508 RSD (≈ 72 €) [9, 10]. This information might explain the low amount of money the participants had at their disposal. The financial aspect is an ongoing issue when dental healthcare comes to mind. Serbian public dental clinics do not charge only for emergencies, such as trauma, swelling, and dental complications directly impacting oral health. That means all other cases are obligated to pay for dental treatment. Not being able to afford dental treatment might lead to deterioration of oral health and, later on, to a state of a dental emergency. All of that could be avoided by introducing adequate preventive measures. Various studies conclude that preventive measures are crucial when preserving good health among migrants and refugees is the aim [11].

The findings gathered in this study show that drinking alcohol among refugees and migrants in Serbia is infrequent. Only 13.3% of participants answered they consumed alcohol, whereas research results from 2006 showed 31.6% of adults in Serbia consumed alcohol 30 days prior to participating in the research [12]. Alcohol consumption is uncommon among refugees and migrants in Belgrade since most of them come from Muslim countries where alcohol consumption is forbidden. A study conducted in Germany found a strong correlation between alcohol consumption and maximal periodontal pocket depth. In addition, smoking and maximal periodontal pocket depth were significantly associated [13]. The data obtained in this study show that 48% of refugees and migrants consume tobacco. Unfortunately, research conducted by the Institute of Public Health of Serbia in 2016 affirms that many adults in Serbia share the same bad habit (38%) [14]. Consuming tobacco, especially smoking, is known to increase the risk of periodontal disease, bad breath, tooth discoloration, delayed healing of intraoral wounds, different types of oral carcinoma, and many more [15]. People who consume tobacco are prone to having various oral health issues and should, therefore, be prioritized as high-risk patients. A public health strategy could be effective in educating and early screening both tobacco and alcohol consumers.

Nutrition is a key factor in general health. Malnutrition and vitamin D deficiency especially have been identified among migrant children in northern parts of the WHO European Region [4]. Findings obtained show that more than one-third of the participants consume sweets on a daily basis. Various studies have shown the side effects of sweetened juices on oral health [16]. The most common effect of high consumption of added sugars on oral health is a greater prevalence of dental caries but also periodontal disease. Both dental caries and periodontal disease are major public health problems globally and are widespread non-communicable diseases. Addressing these health issues and preventing them is of high importance. With significantly more male participants drinking sweetened juices, the findings obtained in this research concur with a study from Udaipur [17]. Our study shows that almost half of the participants consume fruits daily. The results of our study on average fruit intake are higher than in a study conducted in Lithuania but lower than in a study conducted in the European Union [18, 19]. Malnutrition can intensify the severity of oral infections and may evolve into life-threatening diseases [20]. Public healthcare should ad-

dress refugees and migrants suffering either from malnutrition or being overweight/obese but also educate refugees and migrants on a balanced and healthy diet.

Findings from our study showed less than half of migrants brushed their teeth two or more times per day. A study conducted in the United Kingdom showed a higher percentage (71.5%) of Pakistani/Bangladeshi brushing their teeth twice a day [21]. Asylum seekers and immigrants that participated in a study in Finland had similar tooth brushing habits. Women (75%) brushed their teeth more often than men (56%). The same study showed that 57.5% of the participants used toothpaste, whereas 79% of participants in our study used some kind of fluoride supplements [22]. The findings showed a large percentage of children brushed their teeth more than once a day (more than 80%) [23]. These findings are much higher than the ones in this study. Recognizing the need for early dental treatment, providing migrants with adequate oral hygiene utensils, and promoting good oral hygiene could highly impact oral and, therefore, general health.

Oral health affects general health by causing considerable pain and suffering. Because of that, people tend to change their eating habits, speech, quality of life, and well-being [24]. That is the reason for the existence of the undeniable connection between general health and oral health. A comparative study found that approximately one-third of the refugees from the Middle East and Africa that participated in the study had regular oral pain [25]. Oral pain is the most common reason for a visit to the dentist, and our research concurs [22, 26]. Postponing dental treatment may lead to the occurrence of higher risk complications and more difficult treatment procedures. Public healthcare systems should strive to promote early dental treatment and emphasize the importance of prevention.

Some of the principles of healthcare in Serbia are based on our solidarity, efficiency, and protection of patients' rights. Free healthcare is provided to all children under 18 years of age and students till the age of 26, as well as people over 65 years and people with disabilities. Refugees and migrants in Serbia have the same rights and are included in the public healthcare system. The law concerning free dental treatment applies to trauma, swelling, etc., only at public dental clinics [4]. A lower percentage of refugees and migrants go to the dentist in Serbia, probably due to financial issues, language barriers, fear of the dentist, and many more. Conclusions from other studies indicate that the financial aspect and the lack of adequate dental insurance is one of the leading issues for not seeking dental treatment [27, 28]. As for language barriers, refugees and migrants must be able to communicate with healthcare workers. Specialized translators should be at the disposal of refugees and migrants at all times when seeking medical treatment.

This study was among the first attempts to tackle the habits, attitudes, and behavior of refugees and migrants in Serbia in relation to oral health. The study had, however, certain limitations. The sample size of this study can be considered a limitation. Since special permissions were necessary to be obtained prior to every visit to the migrant centers, we limited the sample group to two migrant centers situated in Belgrade. In addition to the excluding factors, an element that also impacted the sample size was that not all residents of the migrant centers were at the premises at the time of conducting the interview.

The number of male participants was dominant in comparison to the number of female participants, which could be seen as a limitation. However, the majority of migrants and refugees in Serbia in 2019 were male [2].

The lack of clinical examinations is one of the shortcomings of this study. Patients with decayed, missing due to caries and filled teeth in the permanent teeth (DMFT) and community periodontal index of treatment need (CPITN) oral status were not registered, and this study did not include radiographs.

Obtaining this information about migrants and refugees would further explain how habits, attitudes, and behavior impact the oral health of migrants and refugees. Clinical examinations should be conducted in future studies.

Conclusion

Based on the study findings, we can understand that in order to provide a safer and healthier environment, attempts should be made to educate and motivate refugees and migrants to maintain oral health. The public healthcare system should focus on refugees and migrants as an at-risk population and make a specialized strategy for them. With a large number of refugees and migrants coming every day to Serbia and other European countries, this public health care issue should be prioritized and further analyzed. Early identification of oral health issues may mean less costly procedures, which would be in the best interest of patients needing dental treatment. Health care providers should have in mind the specifics of the migrant population and adjust procedures and treatment to their needs. The ultimate goal is to preserve and improve oral health among refugees and migrants in Serbia.

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Open pelvic fractures – results of a multi-institutional study

Otvoreni prelomi karlice – rezultati multi-institucionalne studije

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Abstract

Background/Aim. Open pelvic fractures are devastating, rare injuries with high mortality. Leading causes of mortality are the following: hemorrhage, infection, and associated injuries. The aim of this study was to point out methods of treating these injuries and a great number of prognostic mortality factors. **Methods.** In the period from January 2011 to December 2015, 221 patients with pelvis ring fractures were treated at three large clinical centers in Serbia, of which 13 (5%) patients had an open fracture type. We have classified pelvic ring fractures according to the Young-Burgess classification. We have classified injuries according to Gustilo at I, II, and III degrees, and the location of the wound according to Faringer classification was distributed in zone I, II, and III. Urogenital and intra-abdominal injuries were monitored, and the severity of injuries was determined according to Severity Score Injury (ISS) and Trauma Score (TS). **Results**. There were 6 (46%) women and 7 (54%) men with an average age of 41 year (13–76). Injuries from traffic trauma were dominant. The most common causes of pelvic ring fracture were antero-posterior compression – 6 (46%), lateral compression – 4 (31%), and vertical force in 3 (23%) patients. Dominant injuries were types I and II according to Gustilo and zone I according to the Faringer classification. There were 6 (46%) patients with urogenital injuries and the same number with intra-abdominal injuries, of which 3 (23%) patients had been treated with colon resection and diversion. Due to abundant hemorrhage and hypovolemic shock, two patients died, and another one died after three days due to sepsis and multisystem organ failure. **Conclusion.** Open pelvic fractures have a high mortality rate due to: hemorrhage, infection, associated abdominal and genitourinary tract injuries, ISS > 25, TS < 8, and the age of patients > 65 years.

Key words:
fractures, open; injury severity score; mortality; orthopedic procedures; pelvis; risk factors.

Apstrakt

Uvod/Cilj. Otvoreni prelomi karličnog prstena su retke razorne povrede, sa velikom smrtnošću. Vodeći razlozi smrtnosti su: krvarenje, infekcija i udružene povrede. Cilj rada bio je da se ukaže na metode zbrinjavanja tih povreda i veliki broj prognostičkih faktora smrtnosti. **Metode.** U periodu od 2011. do 2015. godine, lečen je 221 pacijent sa prelomom karličnog prstena u tri velika klinička centra Srbije, a 13 (5%) pacijenata imalo je otvoreni tip preloma. Prelomi karličnog prstena su klasifikovani prema Young-Burgess klasifikaciji. Povrede smo podelili prema Gustilo klasifikaciji na povrede I, II i III stepena, a lokalizacija im je određena prema Faringer kalsifikaciji u zone I, II i III. Praćene su urogenitalne i intraabdominalne povrede, a težina povreda određivana je prema *Severity score injury (ISS)* i Trauma skoru (TS). **Rezultati.** Istraživanjem je obuhvaćeno 6 (46%) žena i 7 (54%) muškaraca prosečne starosti od 41 godine (13–76). Dominirale su povrede zadobijene u saobraćajnim udesima. Najčešći načini nastajanja preloma karličnog prstena bili su antero-posteriorna kompresija – 6 (46%), lateralna kompresija – 4 (31%) i vertikalana sila kod 3 (23%) bolesnika. Dominirale su povrede tipa I i II po Gustilo klasifikaciji i zona I po Faringer klasifikaciji. Bilo je 6 (46%) pacijenata sa urogenitalnim povredama, a isti broj i sa intra-abdominalnim povredama, od kojih je kod njih 3 (23%) urađena resekcija kolona. Zbog obilne hemoragije i hipovolemijskog šoka umrla su 2 pacijenta, a još jedan posle tri dana, zbog sepse i multisistemskog organskog poremećaja. **Zaključak**. Otvoreni prelomi karlice imaju veliku stopu smrtnosti, a tome doprinose: krvarenje, infekcija, prateće povrede abdomena i genitourinarnog trakta, ISS > 25, TS < 8 i starost > 65 godina.

Ključne reči:
prelomi, otvoreni; povrede, indeksi težine; mortalitet; ortopedske procedure; karlica; faktori rizika.

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Introduction

Open pelvic fractures (OPFs) represent one of the most devastating injuries in orthopedic trauma. OPF is defined by communication to the lesion of integument, gastrointestinal or urogenital tracts, i.e., direct communication between fracture and the external environment (through the rectum, vagina, or skin) [1, 2]. They are usually the result of extensive force trauma and are associated with multiple injuries. Most often, they occur in traffic accidents, motorcycle drivers, and as a consequence of falling from height.

Fractures of the pelvis are reported to represent 3% of all fractures, with an associated mortality rate from 10 to 16% [3]. OPFs occur in 2–4% of all pelvic fractures, and mortality is high – until 1980, it was up to 50% due to early hemorrhage and late sepsis [3–5]. Today, there are standard resuscitation protocols (Advanced Trauma Life Support – ATLS), which include permanent airway and cervical spine control, good ventilation, and circulation [6, 7]. With this procedure, mortality is reduced to 5–25%. Mortality is not the result of pelvic fractures but of joint injuries – urogenital injuries (23% to 57%), intra-abdominal injuries (up to 50%), and head injuries (up to 35%) [8, 9]. There is a high risk of pelvic sepsis and hemorrhage in these injuries.

Determining whether hemorrhage arises from abdominal trauma, fractured bone surfaces, or ruptured pelvic vessels can be difficult. Hemorrhagic shock is the most common cause of death in the first 24 hrs [2].

Treatment of OPFs conventionally has four critical elements: control of hemorrhage, treatment of soft tissue wound, prevention and treatment of subsequent sepsis, recognition and treatment of associated injuries, and treatment of the fracture itself. Soft tissue injuries should be adequately treated with extensive debridement and rinsing, along with antibiotic therapy, while performing open reduction and stable fixation at the same time, thus preventing infection development and hemorrhage [10, 11].

The aim of this study was to present characteristics, modality of treating these injuries and examine prognostic factors for mortality of patients with OPFs.

Methods

The study included patients treated and monitored in three different centers: the Clinic for Orthopedics and Traumatology in Niš, the Clinic for Orthopedics and Traumatology at the Military Medical Academy in Belgrade, and the Clinic for Orthopedics and Traumatology in Novi Sad. From January 2011 to December 2015, 221 patients with pelvic fractures were directed to our trauma centers, while 13 (5%) of them had an open fracture type. The following data were observed: patient age, sex, injuries, type of pelvic bones fracture, extent and location of soft tissue injuries, orthopedic treatment of fractures and wounds, joint urogenital and abdominal injuries, long bones fractures, Injury Severity Score (ISS), Trauma Score (TS), number of transfusions in the first 24 hrs, and mortality.

Patients were treated in trauma rooms according to Advanced Trauma Life Support (ATLS) guidelines which means permanent airway and cervical spine control, good ventilation, and adequate circulatory support. When patients were in severe hemorrhagic shock, principles of damage control resuscitation were applied. Systolic blood pressure, presence of shock on arrival, and base deficit were all significant predictors of hemodynamic instability. Hemodynamic stability was achieved first by aggressive resuscitation with intravenous fluids and blood products, including clotting factors. Pelvic fracture instability increases hemodynamic instability, so we immediately checked initial stability – positioning and leg rotation, traction, or connecting pelvic ring. If these methods and measures of resuscitation do not achieve hemodynamic stability, it is necessary to gain early stability of the pelvis – provisional stabilization of the pelvic ring that can be achieved either by the application of an external fixator frame or the pelvic C-clamp.

In order to determine the type of pelvic ring fracture, we initially applied an antero-posterior pelvic radiograph. If the patient is in stable hemodynamic condition, additional inlet and outlet pelvic radiographs will help identify pelvic ring disruption and associated displacement. If a patient with OPF is stable, computed tomography (CT) is applied to exclude abdomen injuries and show the spatial position of pelvic fragments.

When it comes to injury mechanism, pelvic ring fractures were classified according to Young-Burgess classification [12]. For the classification of OPFs in relation to stability and rectal injuries, we used the Jones classification [13, 14]. For pelvic fracture stabilization, the method of external, internal, or combined fixation was used. Hospitalization to surgery time differs and depends on patient stability, localization, and condition of injury; surgery was performed in 10–12 hrs to 14 days, and on average, after four days. In extremities fractures, stabilization was applied at the same time, and sometimes even later, depending on the general condition of the patient.

The extent of injury in OPFs was classified according to Gustilo et al. [15]. Surgical treatment of open pelvic wounds included extensive irrigation and debridement of traumatized and devitalized soft tissues. If the condition of the wound allows, delayed secondary wound closure may follow. If there is a great loss of soft tissue, infection, and great soft tissue necrosis when the wound is treated, a delayed skin graft should be applied.

Due to the massive forces applied to cause this injury, most fractures were grade I and II open fractures. All patients with open fractures received tetanus prophylaxis and antibiotics. Furthermore, the location of soft tissue injury in OPFs can be classified as zone I (perineum, anterior pubis, medial buttock, posterior sacrum), zone II (medial thigh, groin crease), or zone III (postero-lateral buttock, iliac crest) Faringer's classification [16] (Figure 1).

Urogenital injuries in OPFs were detected by inspection of external genitalia (labia, penis, scrotum). We monitored bleeding of the external urethral meatus, the

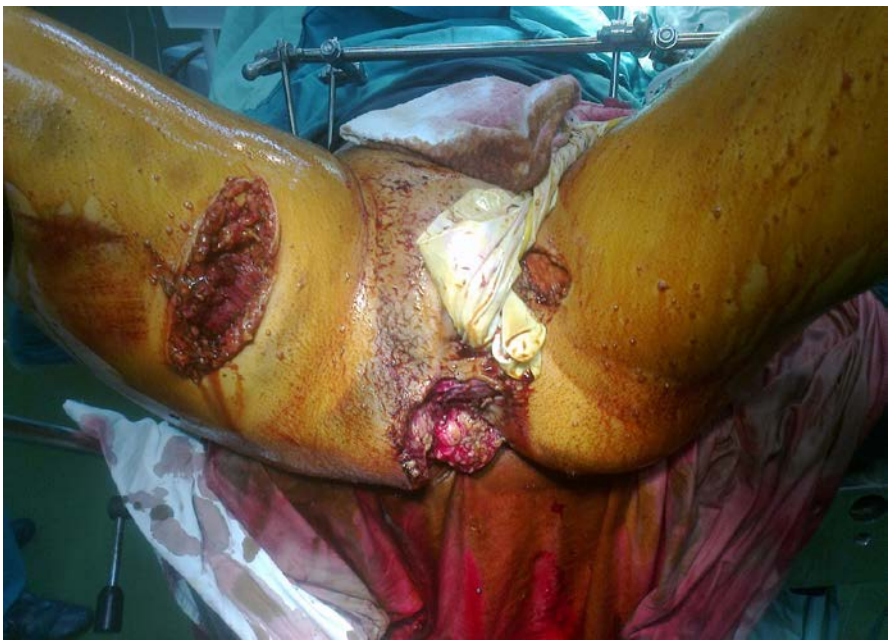

Fig. 1 – Soft tissue injuries associated with open pelvic fracture (Faringer zone injuries I and II).

ability to urinate, and the color of the urine; we examined the perineum, vagina, and prostate. If there is a large displacement of the anterior part of the pelvis ring, there is a suspicion of partial or complete rupture of the urethra, and if possible, a catheter should be introduced. If there is a large intestine injury and perianal wound, colon resection and derivation should be applied, and the wound irrigated periodically, devitalized, and necrotic tissue removed until conditions are met for secondary suture.

During the monitoring period, an ultrasound study of the abdomen was performed in order to detect intra-abdominal bleeding. Hypotensive patients with a positive ultrasound study were indicated for diagnostic peritoneal lavage (DPL). It was applied via a supraventile entry point in order to minimize the possibility of piercing pelvic hematoma and producing a false positive result. If the DPL were grossly positive (> 8 mL of blood aspirated on entry into the peritoneum), operative exploration was indicated [17].

Determining the severity of polytrauma is one of the crucial factors for determining priority in managing injured patients, whether at the injury site or trauma centers. Nowadays, in order to successfully resolve this difficult problem, we have several scoring systems available – anatomical, physiological, or combined. We used the ISS in our study; it gives a numerical description of injuries within the polytrauma and is a type of anatomical scoring system. According to this scale, the body is divided into 6 regions, and with the increase in points, mortality [18] increases. We also used TS, a physiological scoring system. It consists of the Glasgow Coma Scale (GCS), which is reduced by one-third of the value, and an assessment of cardiopulmonary functions. It is composed of 5 parameters, and the number of points is 1 to 16; the higher the score, the greater the possibility of the polytraumatized patient's survival [19].

Statistical analysis

Kolmogorov-Smirnov test for small samples with a marginal value of $p < 0.05$, D = 7, was used for statistical data processing on a small sample of 13 subjects.

Results

In the period from January 2011 to December 2015, 221 patients with a pelvic ring fracture were monitored in these three orthopedic traumatology clinics, of which 13 (5%) patients had an open fracture type. Demographic data of patients, trauma scoring, and mechanism of injury are listed in Table 1.

Table 1

Demographic data, trauma scoring, and mechanism of injury

Characteristics	Value
Age (years), mean (range)	41 (13–76)
Sex, n (%)	
male	7 (54)
female	6 (46)
ISS, mean (range)	30.7 (10–69)
TS, mean (range)	10.8 (4–16)
PRBCs (first 24 hrs), mean (range)	7 (0–18)
Mechanism of injury, patients n (%)	
pedestrian struck by a car	5 (39)
motorcycle collision	3 (23)
fall	3 (23)
tractor driver	2 (15)

ISS – Injury Severity Score; TS – Trauma Score; PRBCs – Packed Red Blood Cells.

The most common cause of pelvic fracture was antero-posterior compression (APC) in a total of 6 (46%) patients.

Lateral compression (LC) as a mechanical fracture factor was noted in 4 (31%) and vertical force (VS) in 3 (23%) patients.

The radiographic findings of patients with open pelvic fractures are shown in Figure 2.

The number of patients in the first open pelvic ring fractures group (stable OPFs) of patients, the second (unstable OPFs without rectal injury), and the third group (unstable OPFs in combination with rectal injury) is presented in Table 2.

The magnitude of the injury was classified using the Gustilo classification. There were 4 (31%) patients with type I, 7 (53%) with type II, and 2 (16%) with type III.

The location of soft-tissue injury was classified according to the Faringer system. In 9 (68%) patients, the wound was located in zone I, in zone II in 2 (16%) patients, and in zone III in 2 (16%) patients.

Hemodynamic instability at reception was registered in 9 (69%) patients. The average transfusion requirement for the first 24 hrs was three units of packed red blood cells.

In 6 (46%) patients, urogenital injuries were registered, of which 4 were women and 2 were men. In one girl, vaginal laceration and uterine amputations were found, as well as intraperitoneal bladder rupture. The other three women had lacerations of the vagina – one had intraperitoneal bladder rupture, the second one had extraperitoneal, and the third one had urethra rupture. In two men, a rupture of the urethra was found. Three women were immediately operated on when reconstruction of the vagina and urinary tract was performed, and the fourth died 8 hrs after admission. Both male patients with urethral disruption required suprapubic drainage and subsequent delayed repair. The mortality rate of patients with associated urogenital injuries was 33% (1 of 3 patients).

In this series, 6 (46%) patients were diagnosed with intra-abdominal injuries. They all had laparotomy performed for various reasons: intraperitoneal bladder rupture was found in two women, and besides that, in one of them, a colon serosal tear requiring a sigmoid colon resection and diversion was also present, and the other one had a small bowel injury requiring surgical repair; 2 patients required a sigmoid colon resection and diversion, one had liver laceration requiring surgical repair, and one was with splenic laceration requiring splenectomy. The mortality rate of patients with associated intra-abdominal injuries was 33% (1 of 3 patients).

Orthopedic stabilization of OPFs was performed in 11 (85%) patients, and 2 (15%) patients died in the initial resuscitation phase. External fixation was applied in 7 (54%) patients, of which 1 vertically and 6 rotationally

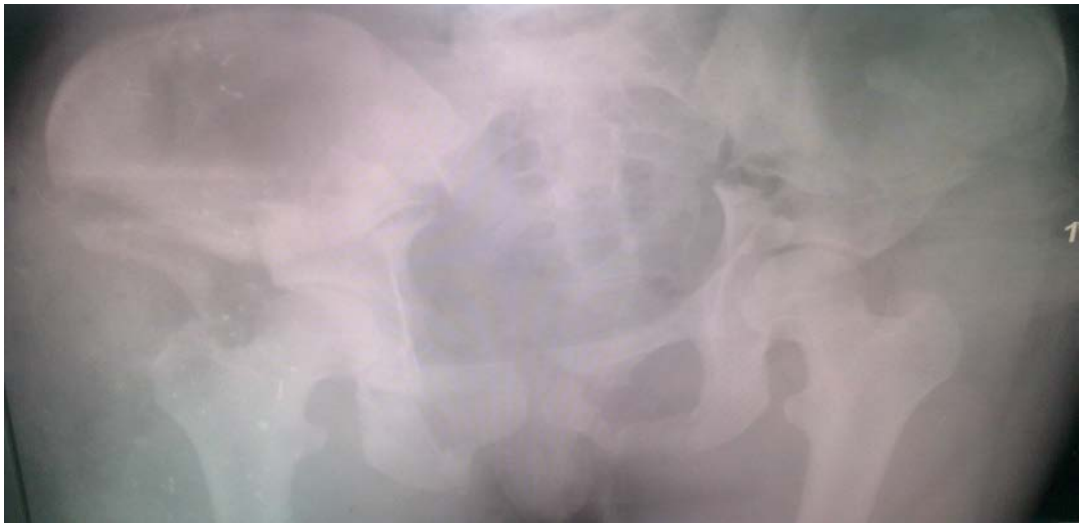

Fig. 2 – Antero-posterior radiograph showing an open pelvic fracture.

Table 2

Type and magnitude of pelvic fractures

Classification	Magnitude	Patients (number)	Toral (percentage)
Young-Burgess			
APC	I	2	46
	II	3	
	III	1	
LC	I	1	31
	II	2	
	III	1	
VS		3	23
Jones			
Group I	I	4	31
Group II	II	4	31
Group III	III	5	38

APC – antero-posterior compression; LC – lateral compression; VS – vertical shear.

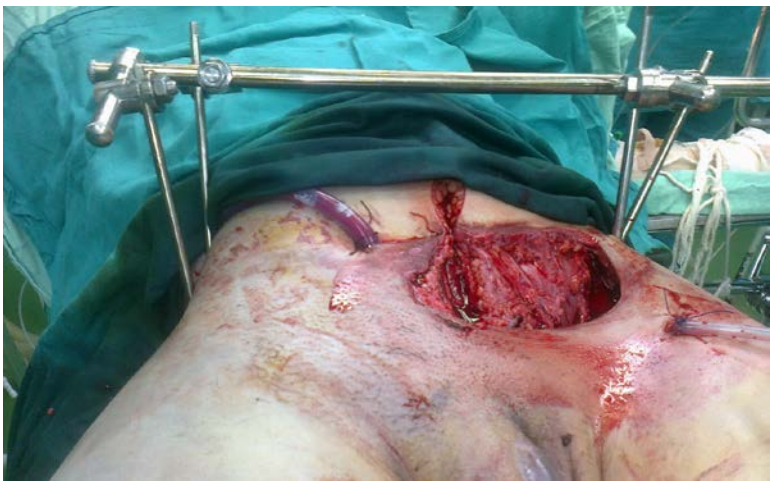

Fig. 3 – An open pelvic fracture stabilized with an external fixator.

unstable fractures. In 3 (23%) patients, internal fracture stabilization was performed, and one had a combination of internal and external fixation – internal femur fracture fixation was applied (Figure 3). After managing wounds in the pelvic region – 15 days on average, a delayed internal fixation was performed in two patients – one vertically and one rotationally unstable fracture and the frontal bow of the pelvic ring was stabilized. Seven patients were treated using the external fixation method, and the apparatus was fixed for seven weeks on average. In 3 (41%) patients, there was a minor infection around pegs – five pegs in total, and it was treated with periodic bandaging. We replaced two pegs of the external fixator in series, i.e., pegs were reinserted in *crista iliaca* because of constant moisture and looseness.

We found no significant correlation between fracture pattern and wound type or location. In 2 (17%) patients, wounds have healed *per primam* – they were located in zone III, in 9 (66%) patients, the wounds have healed *per secundam* with a prolonged period of bandaging and antibiotic therapy, and in 2 (17%) patients, skin graft by Tiersch was applied.

Associated injuries are commonly found with pelvic fractures. The most common joined injuries were fractured extremities – there were five femur fractures – external femur fixation was applied in two patients in the same act when the pelvic ring was fixated, and in three, internal femur fixation was applied with delay after nine days on average**,** and six *cruris* fractures found – external fixator stabilization was applied in four, and in two patients, a lower leg amputation was performed. There were three closed head injuries, one ruptured diaphragm, two pneumothorax, one liver laceration, one small intestine injury, three colon lesions, four perineal and vaginal tears, three urethral injuries, and three bladder ruptures.

Three of 13 patients died (mortality rate 23%), two died in the first 24 hrs due to abundant hemorrhage and hypovolemic shock, and one died after three days due to pelvic sepsis and multisystem organ failure (MSOF). Risk factors for overall mortality are shown in Table 3.

Table 3

Risk factors for overall mortality

Variable	Death		K-S test (D-value)	*p*-values
	yes	no		
Pedestrian struck by a car	2	3		
Motorcycle collision	0	3	D = 7	< 0.05
Fall	0	3		
Tractor driver	1	1		
Young-Burgess class				
APC	2	4		
LC	0	4	D = 7	< 0.05
VS	1	2		
Jones class				
group I	0	4		
group II	1	3	D = 7	< 0.05
group III	2	3		
Gustilo-Anderson grade				
I	0	4		
II	1	6	D = 0	< 0.05
III	2	0		
Faringer class				
zone I	2	7		
zone II	1	1	D = 7	< 0.05
zone III	0	2		
Urogenital injury				
yes	1	5	D = 7	< 0.05
no	2	5		
Intra-abdominal injury				
yes	1	5	D = 7	< 0.05
no	2	5		
Gender				
male	2	4	D = 7	< 0.05
female	1	6		
Age, years				
< 30	1	4	D = 7	< 0.05
> 30	2	6		
ISS				
< 25	0	8	D = 8	< 0.05
> 25	3	2		
TS				
< 8	3	3	D = 7	< 0.05
> 8	0	7		

ISS – Injury Severity Score; TS – Trauma Score; APC – antero-posterior compression; LC – lateral compression; VS – vertical shear.

The results showed there was a statistically significant difference in our material compared to expected values, so there was a greater number of deaths present.

Discussion

OPFs are usually the result of a high energy transfer and are most often seen as part of a trauma mechanism. They most commonly occur in traffic traumatism and are less frequent in case of falling from a height or industrial traumatism [5, 7].

Pelvic fractures are a marker of excessive force applied to the human body and are associated with hemorrhage. Hemorrhage from the cancellous bone surface, the presacral venous plexus, and/or iliac arterial or venous branches can cause hypotension. Hemodynamically unstable pelvic fracture represents a difficult diagnostic and therapeutic challenge for the trauma team. Bleeding is often also extrapelvic due to following injuries (chest 15%, intra-abdominal 32%, long bones 40%). This bleeding is the cause of high mortality in the first 24 hrs – more than 40% [11, 20, 21]. One of the potential causes of late mortality is most likely to be a direct result of the "bloodless vicious cycle" of continuing hemorrhage and transfusion since blood transfusion is an indispensable risk factor for the development of MSOF and death [22, 23]. In our series, 2 (67%) patients died in the first 24 hrs due to hemorrhage, and 1 (33%) died after three days due to MSOF.

Grotz et al. [5] and Bircher and Hargrove [24] have reviewed management priorities in patients with OPFs, including control of hemorrhage, aggressive assessment and management of the wound, stabilizing pelvic ring, early diagnosis of rectal and/or urogenital injuries, and selective use of fecal diversion. That is a protocol for successful OPFs management, which we have also adhered to.

Pelvic ring stabilization is one of the conditions for stopping bleeding and hemodynamic restoration. Biomechanical instability causes hemodynamic instability, i.e., there is a direct correlation. Traditionally, only external fixation techniques were used in OPFs patients [25, 26]. These tools, via external compression, reduce intrapelvic volume and create a tamponade effect against ongoing bleeding. They also restore stability and bone contact to posterior elements of the pelvis and contribute to blood clotting. The pubic symphysis is the weakest link of the structure, representing only about 15% of its stability. Posterior elements – sacroiliac, sacrospinous, and sacrotuberous ligaments – are the strongest, contributing to vertical and antero-posterior stability of the pelvis [27].

In our series, an external fixator was used in 7 (54%) patients. Many authors recommend internal fixation of the pelvic ring in order to achieve both rotational and vertical stability [28]. In 3 (23%) patients, we performed internal fixation of the frontal pelvis ring while performing laparotomy, and in 2 (17%) patients, external fixation was replaced by the inner one, and only after treating wounds and local infection, after 15 days on average.

Hemorrhagic shock is the most common cause of death in the first 24 hrs. In order to eliminate hemorrhage, many authors recommend pelvic packing, i.e., preperitoneal pelvic packing has been suggested to be ineffective for hemorrhage control in OPFs [2, 4, 20, 29]. In our series, this was applied to 4 (31%) patients.

Urogenital injuries are common in OPFs and are a possible cause of developing infection and death. Vaginal lacerations are the result of either penetration of a bony fragment or indirect forces from diastasis of symphysis pubis or bilateral pubic rami fractures [30]. Primary treatment of these injuries is indicated in order to prevent abscess formation. We had 4 (31%) women with vaginal lacerations, of which two had complete uterus amputation up to the cervix. One died 8 hrs after injury, and in 3 patients, revision and bone fragment removal from the vagina were performed. Urethral lesions were conservatively treated by placing a suprapubic catheter. Intraperitoneal urinary bladder ruptures have been operated on, and extraperitoneal have been treated with urethral catheters.

Rectal lacerations with OPF are rare, causing infection, sepsis, and death. Opinions on the method of treatment are opposed. Maull et al. [31], Birolini et al. [32], and Song et al. [14] have operated on all patients and performed total diverting colostomy. The incidence of pelvic infection was lower in patients with early colostomy. Woods et al. [33] and Pell et al. [34] treated fewer patients with this method, treated more patients inoperatively, and found no differences in frequency of infection. In our series, there were 3 (23%) patients with rectal injury, and diverting colostomy was performed on all of them; extensive irrigation and debridement of traumatized and devitalized soft tissues and a secondary seam were applied.

There were 6 (46%) patients with urogenital injuries, 4 women and 2 men. One woman died in the first 24 hrs, and 5 patients were regularly monitored. All three women had dyspareunia (painful sexual intercourse), and the youngest (18 years old) had gynecological surgery in order to apply vagina dilatation and remove the scars; at the time of injury, she had cervix amputation in relation to the vagina. The causes of dyspareunia were: vagina laceration and formation of scar tissue that narrows its lumen, impingement of visceral pelvic organs due to deformation of pelvic ring, as a result of poor treatment of rotational and vertical unstable fractures, and residual displacement of the fracture – more than 5 mm. One male patient was registered with erectile dysfunction of medium level, and the result was pubic diastasis and urethral rupture [35, 36].

The mortality rate in our series of OPFs was 23% (3 deaths out of 13 patients). The mortality rate from closed pelvic fractures was 7% (15 deaths out of 221 patients). Using Fischer's exact test, the difference in mortality was statistically significant for $p < 0.005$. In the study by Hermans et al. [2], no significant difference in survival rate between open and closed pelvic fractures was found. The question was whether the modern improvements in injury treatment, early management, intensive care therapy, damage control, and definitive fracture stabilization techniques would decrease the mortality rate due to pelvic fractures.

Risk factors for OPFs increased mortality include the following: increased ISS, > 25; decreased TS, < 8; age > 65 years; initial systolic blood pressure < 100 mmHg; blood transfusion of > 10 units in 24 hrs; mortality was higher in larger soft-tissue injuries (Gustilo III); location of wounds (Faringer zone I or II); type of bone injury (vertical shear and antero-posterior type); intra-abdominal injury; urogenital injury; pelvic sepsis [37–40]. In our series, the results indicated the same risk factors for mortality in OPFs. A strategic, multidisciplinary response is a critical component in managing these complex and difficult injuries [7, 8, 37].

Conclusion

We presented experiences in the treatment of patients with OPFs in our listed institutions. Vascular damage and bone bleeding associated with pelvic fracture can lead to a very significant, potentially fatal, hemorrhagic shock. OPFs have high mortality due to hemorrhage, infection, and intra-abdominal and urogenital injury. Based on our results, we suggest that ISS > 25, TS < 8, and patients age > 65 years have a poor prognosis on the outcome of treatment.

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Correlation of the expression of tumor necrosis factor-alpha in chronic periapical lesions with the expression of bacterial chaperonin 60

Korelacija ekspresije faktora nekroze tumora-alfa u hroničnim periapeksnim lezijama sa ekspresijom bakterijskog šaperonina 60

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Abstract

Background/Aim. Chronic microbial infections of the root canal are a common issue. This process very often causes an immune reaction in the root canal system that results in forming of chronic periapical lesions (PLs). The aim of this study was to determine the quantitative expression of the bacterial heat shock protein (HSP), chaperonin 60 (*cpn60*), and the pro-inflammatory and anti-inflammatory cytokines in periapical tissue obtained from individuals with chronic PLs and to determine if there is a correlation between the expression of the bacterial HSP and the expression of these cytokines. **Methods.** The study was performed on 18 PLs and 6 control samples of healthy periapical tissue, taken at the Department of Dentistry, Faculty of Medicine, University of Priština/Kosovska Mitrovica. The levels of messenger ribonucleic acid (mRNA) expression of pro- and anti-inflammatory cytokines and bacterial HSP were determined by quantitative real-time polymerase chain reaction (RT-PCR) and quantified by comparing to the internal control gene for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). **Results.** Analysis revealed significantly higher mRNA levels of tumor necrosis factor-alpha (*TNF-a*) and *cpn60* in the tissue of PLs compared with normal periapical tissue ($p < 0.05$). Contrary to these results, the mRNA expression of anti-inflammatory interleukin-10 (*IL-10*) was significantly higher in the samples of normal periapical tissue compared with the mRNA levels of this cytokine in the tissue of PLs ($p < 0.001$). Expression of *cpn60* is in strong correlation with *TNF-a* expression in PLs. **Conclusion.** *cpn60* released from bacteria in periapical tissue could be a strong stimulator of inflammatory response and one of the important players in the pathogenesis of PLs.

Key words:
bacterial outer membrane proteins; chaperonin 60; cytokines; gene expression; heat-shock proteins; interleukin 10; periapical diseases; tumor necrosis factor-alpha.

Apstrakt

Uvod/Cilj. Hronične infekcije korenskog kanala su uobičajen problem. Ovaj proces veoma često izaziva imunsku reakciju u sistemu korenskog kanala, a kao rezultat toga se formiraju hronične periapikalne lezije (PL). Cilj studije bio je da se utvrdi kvantitativna ekspresija bakterijskog proteina „toplotnog udara“ (HSP), šaperonina 60 (*cpn60*) i pro- i anti-inflamacijskih citokina u periapeksnom tkivu dobijenom od osoba sa hroničnim PL i utvrdi da li postoji korelacija između ekspresije bakterijskog HSP i ekspresije ovih citokina. **Metode.** Istraživanje je sprovedeno na 18 PL i 6 kontrolnih uzoraka zdravog periapeksnog tkiva koji su uzeti na Odeljenju za stomatologiju Medicinskog fakulteta Univerziteta u Prištini/Kosovskoj Mitrovici. Nivoi ekspresije informacione ribonukleinske kiseline (iRNK) pro-inflamacijskih i anti-inflamacijskih citokina i bakterijskog HSP određeni su metodom lančane reakcije polimeraze u realnom vremenu (RT-PCR) i kvantifikovani su poređenjem sa internim kontrolnim genom *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*). **Rezultati.** Analize su pokazale značajno više nivoe iRNK faktora nekroze tumora-alfa (*TNF-a*) i *cpn60* u tkivu PL u poređenju sa normalnim periapeksnim tkivom ($p < 0,05$). Suprotno ovim rezultatima, ekspresija iRNK anti-inflamacijskog citokina interleukina-10 (*IL-10*) bila je značajno viša u uzorcima normalnog periapeksnog tkiva u poređenju sa nivoima iRNK ovog citokina u tkivu PL ($p <$

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0,001). Ekspresija *cpn60* je u značajnoj korelaciji sa ekspresijom *TNF-a* u PL. **Zaključak.** *cpn60* oslobođen iz bakterija u periapeksnom tkivu mogao bi biti snažan stimulator zapaljenskog odgovora i jedan od važnih aktera u patogenezi PL.

Ključne reči:
protein spoljne membrane bakterija; šaperonin 60; citokini; geni, ekspresija; protein toplotnog udara; interleukin 10; periapikalne bolesti; faktor nekroze tumora alfa.

Introduction

Periapical lesions (PLs) represent a very common pathology in humans that occurs as a consequence of an immune reaction to the microbial infection in the root canal system [1]. The composition of the infiltrating periapical cells (PCs) in PLs includes various cells of innate and acquired immunity, such as neutrophilic granulocytes, macrophages, T lymphocytes, and plasma cells. In addition to these cells, the cells that make up the normal structure of periapical tissue: fibroblasts, osteoblasts, and the epithelial rests of Malassez, play an important role in the pathogenesis of periapical changes, as well [2].

The primary causes of untreated PLs are the most common bacteria that belong to the genera *Campylobacter*, *Enterococcus*, *Eubacterium*, *Fusobacterium*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Propionibacterium*, and *Streptococcus* [3]. Bacterial antigens in periapical tissue trigger cellular and humoral immune response [4].

Heat shock proteins (HSPs) are synthesized in both prokaryotic and eukaryotic cells and represent the basic cellular defense response to various stressful situations such as fever, bacterial and viral infections, ischemia, hypoxia, radiation, and malignant transformation [5]. Despite having a protective role, HSPs can be strong stimulators of immune cells due to the high homology with bacterial HSPs and the mechanism of molecular mimicry and could contribute to the development of chronic inflammatory processes [6]. The housekeeping gene that encodes the bacterial protein chaperonin 60 [*cpn60* (synonyms are *groEL* and *hsp60*)] assists proper protein folding in bacterial cells and is ubiquitously distributed among bacteria [7]. Gene *cpn60* is marked as a DNA barcode for bacteria [8]. It has been shown that Cpn60 proteins isolated from different bacteria stimulate immune response and contribute to the activation of lymphocytes [9]. Moreover, in several different studies, it has been shown that Cpn60 proteins stimulate the production of cytokines that play the main role in tissue destruction and bone resorption in periodontal disease [10].

Pro-inflammatory and anti-inflammatory cytokines are present in PLs, and the development of PLs depends on their mutual relationship [11, 12]. Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine whose expression is increased in PLs and has a stimulating effect on PL progression and bone destruction [13]. It is produced by macrophages, fibroblasts, monocytes, T and B lymphocytes, and large amounts of this cytokine are released under the influence of bacterial products [13]. By contrast, interleukin-10 (IL-10) belongs to the group of anti-inflammatory cytokines and has a suppressive effect on the development of PLs and bone destruction [14]. In PLs, the main source of IL-10 is B and T lymphocytes, fibroblasts, and macrophages [15]. During the acute inflammatory response, IL-10, in combination with transforming growth factor-beta (TGF-β), suppresses the immune response and promotes tissue repair [14, 15].

The aim of this study was to evaluate the quantitative mRNA expression of the bacterial HSP *cpn60* and pro-inflammatory and anti-inflammatory cytokines in the periapical tissue obtained from individuals with chronic PLs and to evaluate the correlation between the expression of bacterial HSP and the expression of these cytokines.

Methods

Human subjects

Samples of PLs (n = 18) were taken from individuals aged 18–65 years who had their diagnosis of chronic PLs made based on the anamnestic data, clinical manifestations, and X-ray findings. Samples were taken during dental extraction or apical surgery. The control sample (n = 6), obtained from the healthy periapical tissue, was taken during the extraction of healthy teeth for orthodontic purposes. All participants were free of systemic diseases and free of antibiotic therapy during the month that preceded the intervention compared to the sampling period. Participants with PLs were included in the study from the total population of patients treated at Department of Dentistry, Faculty of Medicine in Kosovska Mitrovica, after being examined by an oral surgeon. The control group was selected after the orthodontist's check-ups and after defining the indications for tooth extraction. This study was approved by the Ethics Committee of the Faculty of Medicine, University of Priština/Kosovska Mitrovica (No: 09-537-1). All patients signed a written informed consent form prior to all the procedures. The study was conducted in full accordance with ethical principles. The inclusion of all samples was carried out successively, according to the principle of the natural sample.

Sample collection

After tooth extraction, PLs were removed from the root tip with a sterile scalpel, while during an apicotomy procedure, a sample of the PL was taken by removing the PL of the bone with a curette [12]. The samples were soaked in sterile saline and dried with sterile gauze. The samples were stored in sterile Eppendorf tubes containing RNAlater™ Stabilization Solution (Thermo Fisher Scientific) at -20 °C until RNA extraction.

RNA extraction

Total RNA was extracted from 100 mg of each sample with TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's recommendations. Briefly, periapical tissues were homogenized in 1 mL of TRIzol reagent, centrifuged at 12,000 xg at 4 °C for 10 min, and supernatants collected. Bromochloropropane (Sigma Aldrich) was added to supernatants, and after incubation at room temperature, samples were centrifuged at 12,000 g at 4 °C for 20 min. The aqueous phase was collected, and RNA was precipitated by isopropanol. Samples were washed twice with cold 70% ethanol. The samples were dried and dissolved in RNase-free water.

Real-time Polymerase Chain Reaction

Complementary DNA was synthesized using 1 mg RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) in the presence of oligo (dT) primers. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using Power SYBR MasterMix (Applied Biosystems) and mRNA-specific primers for *TNF-a, IL-10,* and bacterial *cpn60,* while glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as a housekeeping gene for normalization (Table 1). qRT-PCR reactions were initiated with a 10-minute incubation time at 95 °C followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s in a Mastercycler® ep realplex (Eppendorf, Hamburg, Germany). For each sample relative amount of mRNA was normalized to an endogenous reference gene *GAPDH*. Results were obtained as threshold cycle (CT) values. Fold expression changes were calculated with the ΔΔCT method [16]. The expression levels of mRNAs were expressed as the ratio and mean ± standard error (SE) of each specific primer to *GAPDH* expression.

Statistical Analysis

Data analysis was performed using SPSS Statistics for Windows software (version 22.0; SPSS, Chicago, IL). Data were subjected to the Shapiro-Wilk test to characterize their normality, and statistical significance was determined by the Mann-Whitney *U* test. All *p*-values less than 0.05 were considered significant.

Results

The levels of mRNA expression of pro- and anti-inflammatory cytokines and bacterial HSP were determined by RT-PCR and quantified by comparison with the internal control gene *GAPDH*. Analysis revealed significantly higher mRNA levels of *TNF-a* and bacterial HSP, *cpn60,* in the tissue of PLs compared with normal periapical tissue ($p < 0.05$). Contrary to these results, the mRNA expression of anti-inflammatory *IL-10* was significantly higher in the samples of normal periapical tissue compared with the mRNA levels of this cytokine in the tissue of PLs ($p < 0.001$) (Figure 1).

Table 1

Primer sequences of analyzed genes

Gene	Sense and antisense primers
TNF-a	5′-CCAGGCAGTCAGATCATCTTC-3′ 5′-GTTATCTCTCAGCTCCACGC-3′
IL-10	5′-ATG CCC CAA GCT GAG AAC CAA GAC CCA-3′ 5′-TCT CAA GGG GCT GGG TCA GCT ATC CCA-3′
cpn60	H279: GAIIIIGCIGGIGAYGGIACIACIAC H280: YKIYKITCICCRAAICCIGGIGCYTT
GAPDH	5′-TGGAAGGACTCATGACCACA-3′ 5′-AGGGGTCTACATGGCAACTG-3′

***TNF-a* – tumor necrosis factor-alpha; *IL-10* – interleukin 10; *cpn60* – chaperonin 60; *GAPDH* – glyceraldehyde 3-phosphate dehydrogenase.**

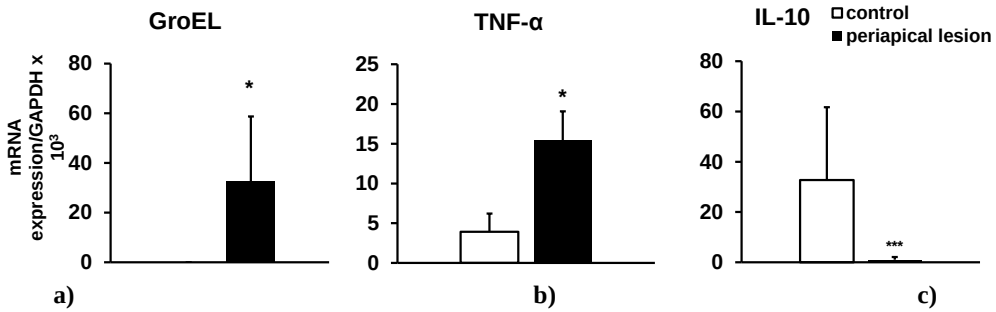

Fig. 1 – Relative mRNA expression levels for: bacterial HSP, *groEL* (*cpn60)* (a), *TNF-a* (b) and *IL-10* (c) in healthy periapical tissue (control) and tissue of periapical lesions determined by qRT-PCR. Data were normalized to the expression of the endogenous control gene, *GAPDH*. Relative expression is calculated by using the ΔΔCT method and presented as mean ± SE. **p* < 0.05, ****p* < 0.001. mRNA – messenger RNA; *TNF-a* – tumor necrosis factor-alpha; *IL-10* – interleukin 10; HSP – heat shock protein; *cpn60* – chaperonin 60; qRT-PCR – quantitative real-time polymerase chain reaction; *GAPDH* – glyceraldehyde 3-phosphate dehydrogenase; SE – standard error.

Table 2

Correlation analysis between relative gene expression levels of the analyzed genes in periapical tissue lesions

Gene		*TNF-α*	*IL-10*	*cpn60*
TNF-a	r	-	0.195	0.632**
IL-10	r	0.195	-	0.099
cpn60	r	0.632**	0.099	-

***TNF-a* – tumor necrosis factor-alpha; *IL-10* – interleukin 10; *cpn60* – chaperonin 60; r – Pearson correlation coefficient.**

****Positive correlation, significant.**

A significant positive correlation was observed between the expression levels of pro-inflammatory cytokine *TNF-a* and mRNA expression level of bacterial HSP, *cpn60* (Table 2), a well-known marker of bacterial contamination [17].

Discussion

This is the first study investigating the quantitative expression of bacterial HSP *cpn60* in the tissue of chronic PLs. Here we have shown for the first time that bacterial *cpn60*, detected by the universal target for bacterial HSP detection, degenerate PCR primers for the amplification of a 549-567 bp region of *cpn60* corresponding to nucleotides 274-828 of the *Escherichia coli* [18, 19], that it is highly expressed in PLs, and that its expression highly correlates with expression of the pro-inflammatory cytokine *TNF-a*.

In apical periodontitis, microorganisms release various molecules that stimulate the innate immune response and thus cause inflammation and tissue damage, regardless of their virulence and tissue invasiveness [20]. HSPs, in addition to their protective role, are also known to have potential to significantly influence the development of the immune response [9].

The expression of human HSPs [21], which are thought to play a role in the pathogenesis of PLs and affect the course of various odontogenic lesions [22, 23], has been established in PLs, but the expression level of bacterial HSPs in PL tissue, has not been examined so far. The negative finding of HSP60 in the epithelial rests of Malassez, and the increased level of HSP60 expression in the epithelium of radicular cysts, also indicate the connection of HSPs with inflammatory processes in PLs [24]. There are also grounds for determining the connection between the expression of certain HSPs and the genetic susceptibility for the development of PLs [25]. The proliferating and cytotoxic activities of *Aggregatibacter actinomycetemcomitans's* GroEL protein on epithelial cells were also reported [26]. In this study, we found *cpn60* expression in all samples of PLs and all samples of the control group, but this expression is significantly higher in PLs than in healthy control tissue.

Increased expression of human Cpn60 in periodontitis has been reported suggesting its significant role in immune-related events during the development of periodontal diseases and in the course of the diseases [27]. Hasan et al. [28] reported that bacterial HSP65 induces a stronger proliferation of peripheral blood T lymphocytes in patients with periodontitis compared with the human HSPs. Yamazaki et al. [29] proved a stronger proliferative response of mononuclear cells isolated from peripheral blood of patients with periodontal disease to the human HSP60 compared to the response of mononuclear cells isolated from the blood of healthy individuals, while the proliferative response of peripheral blood cells to the bacterial HSP, GroEL, was not detected.

Literature data indicate that bacterial HSP (GroEL, Cpn60), originating from different bacteria, induces the expression of cytokines in cells of the host's immune system [30, 31]. It is known that chaperonin Cpn60 from *Mycobacterium tuberculosis* induces the production of TNF-α in the human monocyte cell line, THP-1 [32, 33]. TNF-α is a mediator of osteoclastic activity in states of inflammatory osteolysis such as PLs [34]. The expression of pro-inflammatory cytokine TNF-α in PLs has been demonstrated [13]. Bacterial chaperonins are also known as strong activators of osteolytic activity, similar to TNF-α [35].

In addition, cpn60 from *Mycobacterium tuberculosis*, after the stimulation of toll-like receptor 2 (TLR2) on macrophages, induces the production of the anti-inflammatory cytokine IL-10 [36]. The anti-inflammatory activity of IL-10 is well known and is crucial in the control of periodontal diseases and inhibition of bone resorption [37].

Hence, our goal was to explore the correlation between the expression of bacterial chaperonin *cpn60* and two cytokines, *TNF-a* and *IL-10*, both expressed in the tissue of PL but with opposite effects on PL development and progression.

In this study, we have shown a strong correlation between the expression of *cpn60* and *TNF-a* in all samples of PLs. These results correlate with studies that examined the effect of bacterial HSPs on the TNF-α expression [30, 38, 39].

The higher expression of the immunoregulatory cytokine *IL-10* was found in samples of healthy periapical tissue relative to PLs. Although it has been previously shown that *Actinobacillus actinomycetemcomitans's* GroEL protein can induce the production of interferon-gamma (IFN-γ) and IL-10 in T-bet expressing $CD4^+$ T lymphocytes [31] and those human monocytes stimulated with GroEL have increased expression of IL-10 [30], no correlation in expression of *IL-10* and *groEL (cpn60)* in the tissue of PLs was found in this study. Instead, we have found higher expression of IL-10 in healthy tissue in comparison with the tissue of PLs. Recently, an increased expression of IL-10, 7 days after the reduction of the intracanal microbial load, has been observed [14]. Thus it could be possible that lower expression of *IL-10* in the tissue of PLs and the absence of correlation with the expression of *cpn60* is associated with the clinical characteristics of these lesions.

Conclusion

Based on the results obtained from a small number of patients, we can conclude that the expression of the bacterial HSP *cpn60* in the tissue of PLs strongly correlates with the expression of the pro-inflammatory cytokine *TNF-a*, imposing *cpn60* as a stimulator of inflammatory response in periapical tissue and one of the players in the pathogenesis of PLs.

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Clinical trial challenges – impact of the new clinical trial regulation on the conduct of clinical trials

Izazovi u kliničkim ispitivanjima – uticaj nove uredbe na sprovođenje kliničkih ispitivanja

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Introduction

Clinical trials are very important for the development of new therapeutic options. The current high standards in medical practice can be attributed to the large number of clinical trials that have been conducted so far. Without clinical trials, there is no progress in medicine and, more broadly, the survival of humanity. Therefore, the aim is to provide appropriate regulation of clinical trials.

In Europe, the largest number of clinical trials is conducted in Western European countries, and the total number of applications for authorization of clinical trials of medicines [Clinical Trial Application (CTA)] across Europe decreased by 25% in the period from 2007 to 2011 [1]. This allowed linking the issue to the potentially problematic provisions of the current EU Clinical Trial Directive No 2001/20/EC [2, 3]. Due to a certain unfavorable impact of reduced activity in the field of clinical trials on public health, several initiatives were launched to encourage the conduct of clinical trials, and finally, it was time to introduce a new Regulation.

The Clinical Trial Regulation No 536/2014 was published in the Official Journal of the European Community on May 27, 2014 [4]. The Regulation was adopted to fortify Europe's attractiveness for clinical trials and provide a favorable environment for conducting clinical trials, thus facilitating access to new therapeutic methods while promoting the rights and safety of clinical trial subjects. The Regulation will repeal the existing Directive and become applicable in Europe when the Clinical Trials Information System (CTIS) and database maintained by the European Medicines Agency (EMA) become ready for deployment, which should happen towards the end of 2021 [4, 5].

Regulatory development overview

The current essential document for clinical trials application is Directive 2001/20/EC, adopted by the European Parliament and the Council of the European Union on April 4, 2001, and implemented on May 1, 2004 [3]. Prior to the entry into force of the Directive, there were different processes and requirements for clinical trial authorizations in the EU Member States (MS), which resulted in "delays and complications detrimental to effective conduct of clinical trials" in EU [3].

Thus, the Directive was the first attempt to harmonize the process of authorization of clinical trials. It is based on the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects 1964, International Conference on Harmonization (ICH) guidelines, and good clinical practice guidelines drafted in 1990 by the European Commission [3]. The Directive was seen as a step towards greater transparency and making new medicines more accessible to patients without compromising their safety. However, there were concerns expressed soon after its implementation [6]. Even though the Directive must be acknowledged for the numerous benefits it has brought, which are primarily reflected in greater safety of subjects, better communication between sponsors and researchers, as well as greater reliability of data and thus clini-

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cal trial results, its provisions issued many problems [7, 8]. Numerous weaknesses have been identified, including different interpretations of the provisions, different concepts of approval in the MS, divergent assessments regarding the same studies, different timelines, different outcomes, poor reporting concepts, etc. [2, 6, 9]. As a matter of fact, a survey conducted by Applied Clinical Trials (ACT) and SCORR Marketing in 2015, which included individuals from different types of companies, including, in part, drug sponsors, contract research organizations, academic institutions, consultancies, and service providers, confirmed negative attitudes about the impact of the Directive on clinical trials and drug development [10]. When asked whether the Directive affected their organization, the majority of respondents (57%) and 94% of drug sponsors said it did. It turned out that, more than ten years after the implementation of the Directive, only a slim majority believes that the Directive has simplified and harmonized the requirements for conducting clinical trials across the EU (52%) and that the benefits of the Directive outweigh the costs (51%). Additionally, the Directive has been criticized by many sponsor organizations, both commercial and academic, for the enormous increase in administrative burdens and costs it imposes [6, 8, 11]. In particular, the provisions of the Directive have been a financial obstacle to non-commercial trials and the conduct of independent studies [8]. The cause of the problem is considered to be the fact that the primary objective of the Directive was to facilitate commercial studies, and attention was paid to non-commercial studies only at the very end [11]. Numerous obstacles imposed by the Directive, as well as many unresolved issues, have made the Directive arguably the most criticized document in the field of pharmaceuticals. The dissatisfaction of patients, industry, and academic institutions was expressed by creating proposals for improving the regulation of clinical trials [12, 13]. These numerous circumstances have created the ground for a further step forward in regulating the conduct of clinical trials of drugs, and the intention is to make progress in scientific research and industry [14].

Regulation 536/2014 aims to provide a competitive legal environment for the development of new drugs, especially special treatments, for instance, for rare diseases, and it is based on the need to fill regulatory gaps and establish a single framework for authorization of clinical trials that will cover all MS. The Regulation consists of 99 articles, divided into 19 chapters, plus 7 annexes [4]. Unlike the Directive, which had to be transposed into national law, the Regulation will have direct applicability within all EU MS.

Key changes of Regulation 536/2014

The new Regulation is based on three fundamental pillars: harmonization of the procedures for carrying out clinical trials due to the submission of a single e-dossier through a new information system, public disclosure of information obtained from clinical trials to increase trust and reliability, and simplified safety reporting requirements.

The objectives of the Regulation are to protect the rights, safety, dignity, and well-being of subjects, as well as to ensure the reliability and robustness of data obtained from clinical trials, to encourage innovation and facilitate the clinical trial application process, and finally to achieve an appropriate level of transparency.

Scope and definitions

A “clinical study” [4] is defined as any investigation in human subjects designed to: a) detect or confirm the clinical, pharmacological, or pharmacodynamic effects of one or more medicinal products; b) identify adverse reactions to one or more medical devices; c) examine the absorption, distribution, metabolism, and excretion of one or more medicinal products.

A “clinical trial” [4] is a clinical study that meets any of the following criteria: a) the assignment of a particular treatment to a subject is predetermined and does not constitute a common medical practice in an MS; b) the decision to prescribe a study drug is taken together with the decision to include the subject in a clinical study; c) in addition to normal clinical practice, diagnostic procedures and monitoring are applied.

The scope has remained unchanged. Thus, the Regulation, as well as the Directive, does not cover the field of non-interventional studies, nor does it apply to all clinical trials conducted in the EU. However, the new Regulation introduces the concept of low-intervention trials.

“Low-intervention clinical trial” is a clinical trial that uses medical products already covered by marketing authorization, and it is involved with a minimal additional risk compared to clinical practice. Therefore, the medical product is used in accordance with the marketing authorization, or its use is supported by published scientific records [4].

Authorization procedures

The most significant novelty is the introduction of a centralized system for reviewing and approving clinical trials. The Regulation simplifies the approval procedure via CTIS that includes the EU portal and database (EUPD) [4]. The EUPD will replace the existing European Union Drug Regulating Authorities Clinical Trials Database (EudraCT). This new information system will enable the provision of a single dossier and a single submission of the application for experimentation in all MS in which the trial will be conducted.

One of the main features of the new Regulation is a coordinated assessment between Reporting Member State (RMS) and the MS concerned, and, therefore, one single decision. The assessment will be made separately for Part I of the dossier, which represents the scientific section (level of intervention, risk/benefit for subjects, manufacturing and importation for investigated medicinal product, labeling requirements, Investigator's Brochure), and Part II of the dossier, which represents the ethics section (informed consent, subject recruitment, data protection, suitability of investigators and trial sites, damage compensation). The Part I assessment is jointly performed by MS concerned, and the assessment is coordinated by an RMS proposed by a sponsor

and approved by MS. Part II is evaluated at the national level, in each MS concerned individually and independently. There are clear timelines for the validation of the dossier, with an additional extension of deadlines given in case of need for further information. Documentation evaluations for both parts last 45 days, plus allowed clock-stop of up to 31 days (12 for response, 7 for review of responses and completion of reports). When the conclusion on Part I and Part II is finally reached, the MS have 5 days to issue a decision.

The concept of tacit approval is also established if an MS does not provide a response within a certain period.

There is a possibility that the MS does not agree with the opinion on Part I, but the disagreement can be issued only if the clinical trial is considered to be able to lead to the patients receiving inferior treatment compared to the normal practice in that MS, or in the case of infringement of national law, as well as in the case when there are concerns related to the safety of subjects, reliability and robustness of the generated data.

A refusal of an application for approval to conduct a clinical trial shall be issued if the opinion on Part I, Part II, or both is negative or if the national ethics committee has issued a negative opinion for that MS. Additionally, there is a possibility of expiration of the authorization in an MS concerned if no subject has been included in the trial within two years.

Transition period

Once the Regulation becomes applicable, there will be a three years transition period. It implies that both the old and the new application procedure for conducting a clinical trial will be parallel.

During the first year of the transition period, sponsors will be allowed to choose the way they want to submit an application – under the Directive and EudraCT database regime or the Regulation and the new IT platform regime.

In the second and third year of the transition period, all applications must be submitted via the new information system introduced by the Regulation. It is expected that all clinical trials authorized under the regime of the Directive will remain under that system, but if they are not completed by the end of the third year, they will have to be switched to the new system.

After the third year, all clinical trial applications will be governed by the new Regulation.

CTIS functionalities

As already stated above, CTIS enables the submission and management of clinical trial applications through the portal and provides communication between MS during the evaluation process. The database enables the storage of non-confidential information and makes them available to the general public.

This system is managed by the EMA, and the goal is to build interaction between this and other systems that are already under the control of the EMA.

The EMA declared public approval of the methodology and next steps regarding the plan to launch CTIS at a meeting in June 2010. CTIS has been proposed to be put into operation in December 2021 (https://www.ema.europa.eu/en/news/highlights-management-board-june-2020-meeting).

Transparency

One of the objectives of the new Regulation is to increase transparency regarding clinical processes and data in order to build confidence.

Article 81 (4) of the Regulation states that the EU database should be publicly accessible by default, with a few exceptions concerning: the protection of personal data, protection of commercially confidential information, protection of confidential communication between MS related to the evaluation of documentation, providing constructive oversight of clinical trials.

It is also stated that only those applications for which a decision has been made will be published and that all data and documents will be published at the first opportunity (except exceptions), with sponsors having the option to defer the timing of specific data/documents publishing. The specific data/documents include: the Investigational Medicinal Product Dossier (IMPD) quality section, draft assessment reports, names of experts, personal information about sponsor staff, personal information concerning the Marketing authorization holder/applicant, financial agreements between the sponsor and the research site, Suspected Unexpected Serious Adverse Reaction (SUSAR) and Annual Safety Reports.

Safety reporting

Given the great importance of timely and accurate safety reporting, the new Regulation has simplified safety reporting requirements to ensure the highest standards of safety for respondents.

SUSARs are presently submitted separately to all competent authorities and ethics committees of the different MS concerned, where they are assessed separately. The same applies to the Development Safety Update Report (DSUR) for an investigational medicinal product. Under the provisions of the Regulation, the sponsor will submit all SUSARs as well as the DSUR through a dedicated module of the Eudravigilance database managed by the EMA. The EMA will then forward the reports electronically to all MS concerned, and they will participate in the evaluation process.

Under the EU Directive framework, there were no requirements for reporting serious breaches of protocol, while the new Regulation stipulates that such cases should be reported within seven days. Moreover, in addition to SUSARs, it is ordered to report any unexpected adverse events with an impact on the benefit-risk ratio.

Discussion

The Regulation is a very detailed and extensive document that establishes procedures with very clearly defined deadlines. A single submission and a single decision valid throughout the EU will undoubtedly simplify the process of approving and conducting clinical trials. Accelerating these processes will greatly facilitate the work of sponsors, national regulators, and ethics committees, and improved requirements will encourage clinical research.

Additionally, the application of the new Regulation will address multiple capabilities during the planning and designing of clinical trials, their conduction, as well as during the reporting of development steps in the trial. While it is true that this entails changes in terms of roles, responsibilities, and both sponsor staff and systems competencies, there is an opportunity for standardization, process optimization, as well as education and training processes, which overall provides higher standards in medicine testing on humans.

The introduction of the category of low-intervention studies will greatly improve the investigating procedures for medicines used in accordance with the approved Summary of product characteristics, which is not regulated well enough by the current provisions. This could encourage additional trials of authorized medicines and allow those products to be used in the best possible way [15, 16].

The new Regulation is expected to reduce the number of redundant trials if applied adequately [17, 18]. Redundant trials are those trials that investigate issues that may be "answered satisfactorily with existing evidence" [19]. Such trials are considered "unnecessary duplication of research efforts" [20, 21] and pose an ethical problem as they unjustifiably lead to exposing the subjects to risks. For example, increased transparency, as one of the main pillars of the new Regulation, is one of the instruments for reducing wasteful research because it will disable this type of research that occurs as a result of lack of transparency [17]. In addition, increasing the availability of data can support academic research, strengthen the integrity of the clinical trial system, and increase public trust in this system [15, 22]. Public disclosure of data also allows all stakeholders to access new information relevant to current and future research and is believed to be able to contribute to the protection of public health [22–24].

On the other hand, new and amended provisions also raise new concerns that may have a significant impact on the regulation of clinical trials. According to the SCORR Marketing and ACT survey, respondents' opinions were divided regarding the effectiveness of the new Regulation. It was found that 51% believed that the measure would go far enough to address some of the obstacles to doing research in Europe, while 49% were not convinced. As many as 46% of respondents stated that they believed that the new Regulation would not improve the rate of applications for clinical trials in Europe [10].

There are concerns that increased transparency regarding the availability of outcome data together with the availability of data related to trial participants may lead to threats to the privacy of trial participants, errors in the interpretation of clinical trial outcomes due to inadequate data analysis, and the risk of commercially confidential data disclosures [23]. An adequate balance needs to be found between the drawbacks and the advantages, and the aim of the EMA is to find an appropriate solution [24, 25]. It is considered that the trial results should not be made publicly available until a marketing authorization has been granted [26].

Given that the development of clinical research goes in the direction of targeting specific groups of patients, which entails the problem of a potentially small number of subjects in studies, multicenter studies are becoming even more significant as a tool to provide a sufficient number of subjects in such studies [27–30]. The importance of the new Regulation is reflected in the fact that the harmonization of requirements for the conduct of clinical trials across the EU sets the basis for facilitating the conduct of multicenter trials. In addition, there is no doubt that a coordinated assessment of clinical trial documentation introduced by the new Regulation provides a quality foundation for advancing research in the field of rare diseases and global epidemics, as well as innovative therapies [15, 27]. However, the fact that the sponsor will have the right to choose an RMS (with the approval of other MS) raises concerns that only a limited number of countries will act as an RMS due to preference given to individual countries by the sponsor for various reasons [26].

Some provisions require extremely careful planning and synchronization. For instance, appropriate coordination will need to be established between national competent authorities and ethics committees working on assessments in parallel and within a defined timetable [26, 31, 32]. The division of assessment tasks is an effective way to simplify the complex evaluation process but raises the issue of limiting the scope of evaluation of ethics committees only to Part II items due to its simplistic interpretation [33]. As a matter of fact, the new Regulation allows MS to determine the assessment area of ethics committees [34]. This means that they can opt for a model that involves only the assessment of Part II or a model that also includes the assessment of some Part I issues. However, it should be borne in mind that limiting the assessment process to Part II alone may have an impact on the safety of subjects in clinical trials, given the omission of some important elements in the evaluation by ethics committees, such as methodology and risk-benefit ratio. On the other hand, the current Directive requires that ethics committees also consider these aspects of clinical trials [35, 36]. In addition, the envisaged assessment deadlines are shorter in the new Regulation compared to the Directive [36]. Overall, adaptation to the provisions of the new Regulation will have an impact on the work of ethics committees through the introduction of changes in the existing system of evaluation processes by ethics committees. This will give rise to a thorough definition of the functioning and duties of the ethics committee within the legal framework but will also call for reorganization as well as the provision of adequate resources.

One of the issues to consider is the relocation of clinical trial sites outside the EU, particularly in Western Europe. A study by da Silva et al. [37] dealt with the phenomenon of globalization of clinical trials and found that the largest aver-

age increase in the number of clinical trials between 2005 and 2012 occurred in the Asian (30%) and Latin American/Caribbean (12%) regions. It was also found that the largest average annual increase in the number of clinical trials was related to the lower-middle income (33%) and low-income (21%) regions [37]. Reasons for this trend include the burden of bureaucracy and the high costs associated with richer countries. The complexity of the demanding provisions is also considered to be a major burden in terms of compliance, documentation, and training [38]. The new Regulation aims to stimulate the conduct of clinical trials; however, the results of the survey conducted by ACT and SCORR Marketing do not indicate the existence of high reliability in such potential of the Regulation. One of the main points of this survey is the fact that the respondents believe that in the near future, clinical trials will be largely transferred from Europe to Asia and Latin America. It is believed that clinical trials will be conducted to a greater extent in China (chosen by 46% of respondents), other Asian countries, such as Japan or North Korea (according to 40% of respondents), and Latin America (according to 37% of respondents) [10].

Impact of Regulation 536/2014 on the Balkan region

The EU is becoming a more competitive market for clinical trials, including smaller EU countries that have been neglected so far due to long and complicated procedures and lack of human resources, which may reduce the Balkan region's participation in the clinical trial market.

EU members from the Balkan region (Croatia, Slovenia, Romania, and Bulgaria) will have to accept the Regulation without changes and adaptations. Countries in this region that are candidates and potential candidates for membership of the EU (Serbia, Montenegro, Bosnia and Herzegovina, and Macedonia) will have to start adapting their laws in order to be ready to introduce the Regulation without changes and adaptations.

With that in mind, it is necessary to continue to harmonize national provisions with EU regulations. On that occasion, the implementation of the highest ethical and scientific standards related to the approval, conduction, and control of clinical trials should be encouraged. It is also necessary to optimize the deadlines for approving clinical trials, as well as to increase transparency in the process of approving clinical trials.

Steps are being taken to create an appropriate basis for the development of a suitable environment for clinical trial conduction. For example, the Ethics Board of Serbia was set up in Serbia in 2019 [39]. Until then, the ethical aspects of clinical trials were assessed by the ethics committees of individual health care institutions. This approach to the regulation of clinical trials contributes to the shortening of deadlines for the evaluation of documentation and simplifying the decision-making on clinical trials approval and, consequently, to a more efficient framework for medical research involving human volunteers. Meanwhile, in Montenegro, the August 2020 law on medicines provides for the establishment of an Ethics committee of the Ministry of Health whose responsibility is to issue opinions on all clinical trials conducted in Montenegro, including multicenter clinical trials [40]. The implementation of the new progressive provisions in the existing system of clinical trial documentation assessment is a great challenge but also a significant opportunity to increase the number of clinical trials as it will hopefully positively affect the sponsors' perception of clinical trials' conduction in the region.

Conclusion

Overall, the Regulation introduces new important measures that are expected to contribute to the increase in the number of clinical trials in Europe. The Regulation addresses the administrative burdens of the application process caused by redundant bureaucracy as well as the slow approval process. Although the Regulation, unlike the Directive, must be fully incorporated into national legislation, the new requirements are sufficiently broad to provide MS with sufficient flexibility in the implementation process. There are still certain barriers that need to be considered and which can burden regulatory agencies; however, the potential of the Regulation is too large to step back in the face of that challenge. The new provisions raise confidence that clinical trials will benefit greatly from the Regulation if adapted correctly. As the Regulation will have a strong impact on the requirements for clinical trials in Europe, the EMA, MS, and sponsors need to make appropriate preparations to introduce the Regulation into the clinical trial system in the best possible way. The implementation of such large measures implies the inevitable complexity, but this will ultimately result in harmonization across all EU trials and greater efficiency in achieving drug approvals.

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CASE REPORTS

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Bundling method to treat extensive thumb fingertip pulp incisions – a case report

Metoda spajanja za lečenje ekstenzivnih rezova pulpe vrha palca

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Abstract

Introduction. Thumb fingertip injuries, often combined with phalanx fracture or torn ligament, are very common. The focus of treatment is on how to rebuild the function and shape of the thumb. Single thumb fingertip pulp injury is more common. However, due to the diverse type of injury, no uniform treatment guideline is described in the literature. **Case report.** A patient with extensive thumb fingertip pulp incisions, which looked like multiple parallel wounds, was admitted to our Emergency Department. We used the method of bundling to maintain the shape of the fingertip. A single suture to pass through all the incisions was performed and then the suture was tied outside the skin. After that, we bandaged them with sterile bandages. Finally, good contour and fingertip function were restored. **Conclusion.** We successfully used the bundling method to treat patient with extensive thumb fingertip pulp incisions.

Key words:
reconstructive surgical procedures; suture techniques; thumb; wounds.

Apstrakt

Uvod. Povrede vrha palca, često kombinovane sa frakturom falange ili pokidanim ligamentima, veoma su česte. Fokus u lečenju je na obnovi funkcija i oblika palca. Povreda pulpe vrha palca je još češća povreda. Međutim, zbog raznolikosti tipa povreda, u literaturi ne postoje jedinstvene smernice za lečenje takvih povreda. **Prikaz bolesnika.** Pacijent sa povredom tipa opsežne incizije pulpe vrha palca, sa izgledom multiplih paralelnih rana, lečen je na našem Odeljenju urgentne medicine. Da bi održali oblik vrha prsta, koristili smo metodu spajanja. Koristili smo jedan šav da bismo prošli kroz sve rezove, a zatim smo vezali šav izvan kože. Posle toga prst je previjan sterilnim zavojima. Na kraju, prstu je vraćena pravilna kontura i obnovljena je funkcija vrha prsta. **Zaključak.** Uspešno smo koristili metodu spajanja za lečenje pacijenta sa opsežnim rezovima pulpe vrha palca.

Ključne reči:
hirurgija, rekonstruktivna, procedure; šavovi, tehnike; palac ruke; rane i povrede.

Introduction

The fingertips have special anatomical features and highly complex functions. Therefore, once the injury occurs, it brings great inconvenience and disability to the patient [1, 2]. Unfortunately, fingertip injuries occur quite commonly. There is no clear classification of fingertip injury to guide clinicians in proper management. Usually, injuries are defined as defects or no defects. Other definitions involve structures, such as pulp, nails, and bones, which also define the location of the injury. There are many treatment methods in the literature that describe defects and multiple structural composite injuries [3]. However, simple fingertip pulp injury is rarely mentioned. The finger pulp is the main soft tissue that covers the finger, providing an important pinch function and a sensitive touch [2]. Usually, after the fingertip is injured, we simply bandage it with Vaseline® or semi-occlusive dressing after thorough debridement. Undoubtedly, it is convenient to handle simple incisions. However, for complex incisions, a simple dressing may bring deformity healing, and changing dressings could be problematic.

Injuries involving more than one parallel incision or lacerations may require horizontal mattress sutures to cross all incisions to prevent damage to the blood vessel supply of the skin island located between the incisions. As the fingers are cylindrical, if the wound is valgus, it can lead to a crack after tearing multiple thumbs with horizontal mattress sutures. We presented a patient with a severe incision on the thumb and fingertips, which involved multiple parallel wounds.

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Case report

A 52-year-old butcher presented at our Emergency Room with extensive incisions on the fingertip of his right thumb. He accidentally put his right thumb in the meat slicer while working. There were multiple parallel incisions on his right thumb fingertip pulp (Figure 1). The incision did not involve the tendons in zone 1, and the image showed no fracture. Tetanus injection was performed before surgery, and ceftazidime was used to prevent infection. The incision involved the entire fingertip, but not the nail bed, and the distance between the incisions was only about 1–2 mm, and the depth was 3–5 mm. Horizontal mattress sutures were suitable for multiple parallel incisions, although it is limited to cases where the skin is flat and the soft tissue is thick.

After obtaining the patient's consent, we performed debridement in the Emergency Room. After anesthetizing the root of the finger, we used a single suture to pass through all the incisions and then tied the suture outside the skin (Figure 2). The patient left the hospital immediately after the operation. The patient was given oral anti-inflammatory drugs and analgesics and told to come for a follow-up at the clinic. The dressing was changed regularly after the injury, and because there was more wound fluid in the first week, the dressing was changed every day, and every 2–3 days after a week. The thread was removed 14 days after surgery.

The patient's incision healed 2 weeks after surgery, and we removed the tied sutures and wrapped the affected thumb with sterile gauze. At 12 months follow-up, the incisions of the thumb fingertip healed well, and the contour of the fingertips returned to normal (Figure 3). The thumb's flexion and extension function were normal, and there was no abnormal pain in the fingertips but a little numbness.

Fig. 1 – The patient's thumb fingertip pulp was severely injured, with 5 parallel lacerations, but the nail bed was intact.

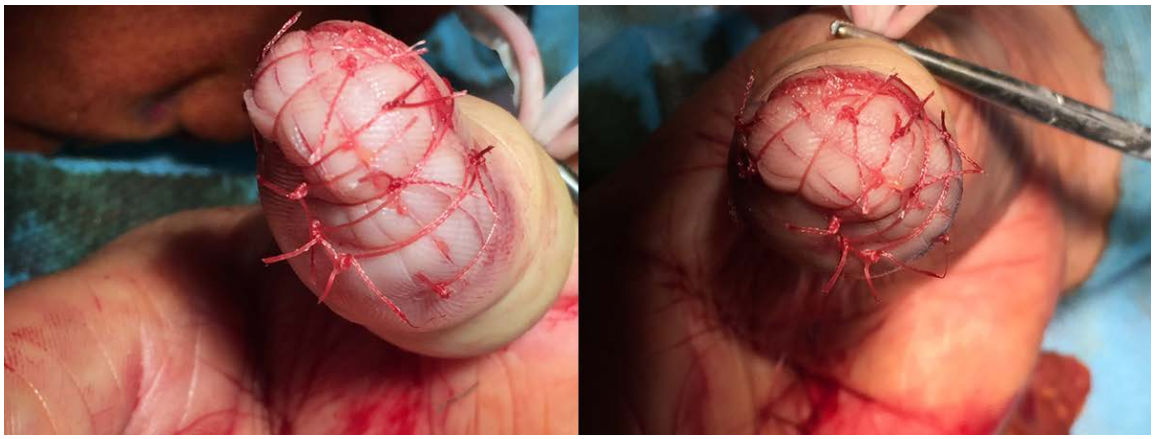

Fig. 2 –After wearing the tourniquet, all the lacerations were crossed with single sutures (left), and the sutures were bundled outside (right).

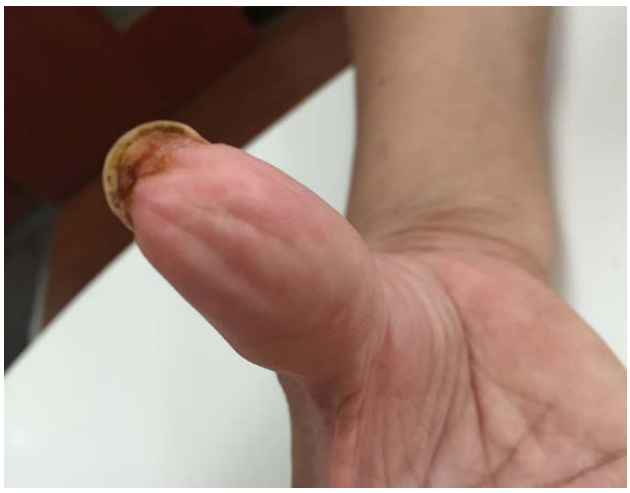

Fig. 3 – After a year of follow-up, the patient's fingertips healed well and there were no hypertrophic scars.

Discussion

Hand injuries are the most frequently encountered injuries, contributing up to 30% of accident and emergency attendances [1]. Fingertip incisions are an important part of hand injuries. The highly specialized structure at the fingertips can achieve tasks requiring precision, strength, and durability. Therefore, fingertip injuries, especially pulp injuries, can affect the entire hand [2]. There is a huge difference in the severity of fingertip injuries, ranging from small incisions involving skin to compound injuries including bones, tendons, and nail beds. Consequently, it is important to determine the mechanism and classification of the injury because it can indicate the degree of contamination, the amount of tissue loss, and the best treatment.

In adults, fingertip injuries are often related to professional activities. Employees of meat packaging are submitted to experience incision injuries of hand [4]. Such injured wounds are often neat and sharp. For deep and complex incisions, such as amputation, hand surgery, or even microsurgeon intervention may be required to obtain satisfactory results [3]. If the injury is superficial, or there is no important structural damage in the case of deep injury, simple wound irrigation, debridement, and wound suture in the emergency department are more appropriate, thus avoiding the need for surgery [5]. Quinn et al. [6] conducted a randomized controlled study to assess the difference in clinical outcomes between sutured incisions and conservatively treated incisions. They declared that hand incisions less than 2 cm long without tendons and fractures, which do not involve nail beds, can be cleaned and dressed without suturing, and similar cosmetic effects and normal activities can be obtained; oral or intravenous antibiotics are also not necessary [6, 7]. For incisions longer than 2 cm or complicated, the condition of tetanus, the pathogenic bacteria that may cause infection, needs to be evaluated before treatment, and the damage to the surrounding structure needs to be explored in order to rule out tetanus. There are four most used methods for closing wounds and they include Staples, Sutures, Steri-Strips, and Sticky Stuff [8]. Clinicians need to choose the appropriate closure method according to the patient's condition; there are many reports in this regard. However, there are no studies on managing extensive thumb fingertip pulp incisions.

The case we presented was a staff member from a butcher's shop who accidentally cut his right thumb with a meat slicer while working. His thumb pulp showed multiple parallel incisions. The standard care for treating such wounds is to vigorously wash the wound, debride, and remove foreign bodies; whether antibiotics should be used prophylactically remains controversial [9]. Considering the patient's working environment, the risk of *Staphylococcus aureus* infection is greater; we gave him an intravenous infusion of antibiotics to prevent possible infections. The distance between the patients' incisions was too close, and thus, choosing the appreciate suture method was hard. Because the skin of the thumb and fingertips is curved, we did not choose the horizontal mattress suture. In addition, we were concerned that the petrolatum gauze covering may cause poor wound healing. Therefore, we decided to use sutures to bind the affected finger and then bandage them with sterile bandages. Considering that binding may cause iatrogenic finger ischemia [10], we checked the blood supply after loosening the tourniquet. Eventually, the patient obtained a complete recovery of the thumb with a satisfying appearance. To the best of our knowledge, this is the first reported case of using a bundle to treat a complex thumb incision.

Conclusion

Fingertip injuries are the most common injuries in the emergency departments, with various types of wounds. Clinicians should choose the appropriate antibiotics to prevent infections based on the pathogenic bacteria that may exist in the patient's working environment. Most importantly, routine exploration should be performed to assess soft tissue damage and contamination. However, there is a risk of over-tightening resulting in finger ischemia which needs careful care. Moreover, paying attention to the blood supply at the fingertips after the operation is also important. In the future, a flexible mesh finger cuff may be developed to treat this type of injury.

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Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

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Large neck metastasis with unknown primary tumor – a case report

Velike metastaze u vratu sa nepoznatim primarnim tumorom

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Abstract

Introduction. Metastatic head and neck carcinoma (HNC) from an unknown primary tumor is defined as a metastatic disease in the neck's lymph nodes without evidence of a primary tumor after appropriate investigation. Multiple national guidelines recommend taking essential steps in diagnostic protocols which involve a detailed clinical exam with radiological imaging, fine-needle aspiration (FNA) biopsy of the cervical tumor, esophagogastroduodenoscopy (EGD) with palatine and lingual tonsillectomy, immunohistochemical staining, and human papillomavirus (HPV) detection. Treatment of HNCs of unknown primary origin involves surgery (neck dissection) with radiotherapy (RT), while some authors recommend chemo-radiotherapy in cases of advanced regional disease. **Case report.** A 44-year-old male was referred to the tertiary medical center because of a large ulcero-infiltrative cervical mass on the right side. Examination of the head and neck and flexible nasopharyngolaryngeal endoscopy was conducted, followed by computed tomography (CT) of the head, neck, and thorax with intravenous contrast. The primary localization of the tumor was not confirmed by these diagnostic methods. An open biopsy of the neck mass established a histopathology diagnosis of metastatic squamous cell carcinoma (SCC). Results of EGD with biopsies and bilateral tonsillectomy were negative for malignancy. Treatment included extended radical neck dissection with reconstruction and postoperative ipsilateral RT. The patient presented with an extensive pharyngolaryngeal tumor five years after the first surgery. Biopsy with histopathology examination confirmed the diagnosis of SCC. **Conclusion.** A structured step-by-step diagnostic approach to identifying the primary site of the metastatic HNC is mandatory. Substantial advances in diagnostics and operative techniques have increased the likelihood of primary tumor identification and detection of the disease's regional and systemic spread. The purpose of adherence to guidelines results in higher overall survival and longer regional disease-free survival in these patients.

Key words:
biopsy; diagnosis; endoscopy, digestive system; head and neck neoplasms; neoplasms, unknown primary; tomography, x-ray computed.

Apstrakt

Uvod. Metastaze u glavi i vratu karcinoma (HNC) nepoznatog primarnog tumora definišu se kao metastatska bolest u limfnim čvorovima vrata bez dokaza o postojanju primarnog tumora nakon sprovedene dijagnostike. Više nacionalnih vodiča preporučuje da dijagnostički protokoli obavezno uključe detaljan klinički pregled sa radiološkom dijagnostikom, iglenu aspiracionu biopsiju (FNA) tumorskih promena na vratu, ezofagogastroduodenoskopiju (EGD) sa tonzilektomijom palatinalnih i lingvalnih tonzila, imunohistohemijska bojenja preparata i detekciju humanog papiloma virusa (HPV). Terapija metastaza HNC nepoznatih primarnih tumora podrazumeva hirurško lečenje (disekciju vrata) i primenu radioterapije (RT), dok neki autori preporučuju hemoradioterapiju u slučajevima uznapredovale regionalne bolesti. **Prikaz bolesnika.** Bolesnik star 44 godine je, zbog pojave ulceroinfiltrativne mase na vratu sa desne strane, upućen u tercijarnu medicinsku ustanovu. Učinjen je pregled glave i vrata i fiberoptička nazofaringolaringoskopija, a potom i kompjuterizovana tomografija (CT) glave, vrata i grudnog koša sa intravenskim kontrastom. Primarna lokalizacija tumora nije utvrđena tim dijagnostičkim metodama. Otvorenom biopsijom tumorske promene na vratu postavljena je histopatološka dijagnoza metastaze skvamocelularnog karcinoma (SCC). Bolesniku je potom urađena EGD sa slepim biopsijama, čiji su rezultati bili negativni na malignitet. Lečen je hirurški, proširenom radikalnom disekcijom vrata sa rekonstrukcijom i postoperativnom ipsilateralnom RT. Pet godina nakon operativnog lečenja, bolesnik se javio na pregled sa manifestacijama masivnog faringolaringealnog tumora. Biopsijom i histopatološkim ispitivanjem potvrđena je dijagnoza SCC. **Zaključak.** U slučaju metastaze HNC nepoznatog primarnog tumora, obavezan je dijagnostički postupak „korak po korak" za identifikaciju lokalizacije primarnog tumora. Značajan napredak u dijagnostici i

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operativnim tehnikama uticao je na povećanje verovatnoće utvrđivanja porekla primarnog tumora, kao i detekciju regionalnog i sistemskog širenja bolesti. Krajnji cilj je poboljšanje ukupnog preživljavanja i produženje intervala bez regionalnih recidiva kod tih bolesnika.

Ključne reči:
biopsija; dijagnoza; endoskopija, gastrointestinalna; glava i vrat, neoplazme; neoplazme nepoznatog porekla; tomografija, kompjuterizovana, rendgenska.

Introduction

Head and neck squamous cell carcinoma (SCC) of unknown primary (SCCUP) origin makes up approximately 2–5% of all head and neck cancers (HNC), although advances in imaging and intraoperative visualization of high-risk subsites have increased the likelihood of identifying the primary site [1]. Identification of the primary site allows for planning of targeted treatment and is associated with improved prognosis and survival outcomes [2]. Multiple national guidelines recommending diagnosis and treatment strategies were presented in the last decade [1–5], but the consensus on the diagnostic workup of head and neck SCCUP has not yet been reached. Important steps in diagnostic protocols involve a detailed clinical exam with radiological imaging, fine-needle aspiration (FNA) biopsy of the cervical tumor, and esophagogastroduodenoscopy (EGD) with palatine and lingual tonsillectomy. New research is focused on determining the role of human papillomavirus (HPV) detection, the use of fluorodeoxyglucose [FDG]-positron emission tomography (PET)/computed tomography (CT) – (PET/ CT) combined with EGD in the detection of the primary tumor, or the use of transoral minimally invasive procedures [6–8].

Treatment of head and neck SCCUPs prioritizes locoregional control. Initial recommendations involve surgery (neck dissection) with radiotherapy (RT) [1, 2, 9]. The importance of chemo-RT is stressed for N2, N3, and metastases with extracapsular extension [1, 10]. Treatment remains heterogeneous and still based on retrospective studies, clinical experience, and institutional policies.

We present a case of SCCUP of the neck to illustrate the importance of a structured diagnostic protocol and appropriate treatment choice in achieving better overall and disease-free survival.

Case report

A 44-year-old male patient was referred to our clinic with a painless large ulcerous-infiltrative cervical mass on the right side. The neck mass appeared four months prior to referral. On admission, the patient did not report any other relevant symptoms in the head and neck region or any comorbidities or allergies. He was a heavy smoker (up to 60 cigarettes a day for 20 years) and frequently consumed alcohol (over 500 mL of spirits a day for over 15 years).

We conducted a complete and careful clinical otorhinolaryngology examination, followed by flexible nasopharyngolaryngeal endoscopy. Clinical findings appeared normal. Prior to hospitalization, CT of the head, neck, and thorax with intravenous contrast was done. Imaging findings indicated nodal metastatic disease in the right neck, with central necrosis, infiltration of adjacent muscles, internal jugular vein, and skin. All parts of the pharynx and larynx were without pathological findings (Figure 1).

Biopsy of the neck mass confirmed histopathology diagnosis of metastatic SCC. The patient underwent EGD with biopsies from the nasopharynx, both tonsils, tongue base, and pyriform fossae, but the results were negative.

Intraoperative findings indicated infiltration of the posterior digastric muscle, jugular vein, sternocleidomastoid muscle, parotid, gland, XI and XII cranial nerves, and brachial plexus. The defect was reconstructed with the pectoral's major myocutaneous flap (Figure 2).

Histopathology findings were consistent with grade III metastatic SCC, confirming the neck lymph node metastases from an unknown primary tumor. According to the Oncological Board's decision, the patient underwent postoperative RT of the neck (from the skull base to below the cricoid

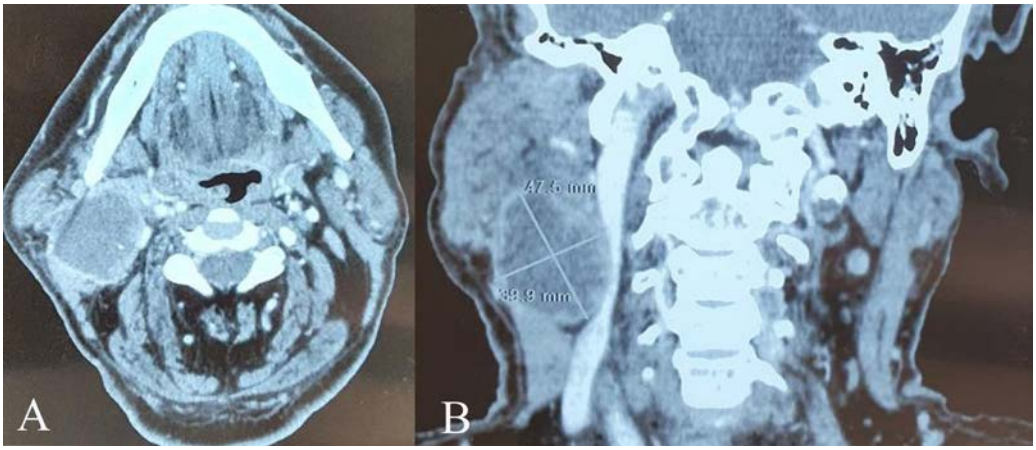

Fig. 1 – Computed tomography (CT) of the patient's neck with nodal metastatic disease in the neck with central necrosis: (A) axial view; (B) coronal view.

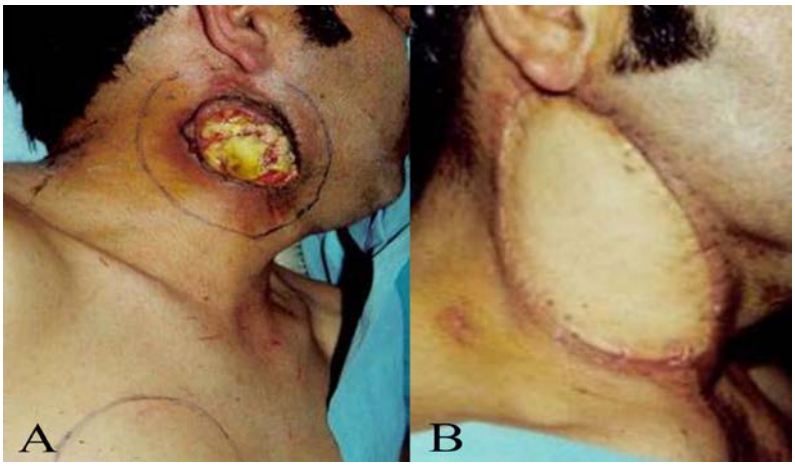

Fig. 2 – Preoperative (A) and postoperative (B) images of the patient metastatic neck mass on the right; extended radical neck dissection was performed and the defect was reconstructed with the pectoral's major myocutaneous flap.

cartilage, including ipsilateral neck level II, III, IV, and parotid gland) with 65 Gy in 30 fractions during six weeks. After three years of regular oncological controls, the patient was lost to follow-up.

Five years after the first surgery, the patient presented with difficulty swallowing. A pharyngeal tumor of the left lateral oropharyngeal and hypopharyngeal wall, with extension to the pyriform fossa and the right larynx involvement, was visualized with indirect laryngoscopy. Biopsy with histopathology examination confirmed the diagnosis of SCC. CT scan of head, neck, and thorax showed no signs of regional or distant disease spread. The patient underwent total pharyngolaryngectomy, tracheostomy, and left selective neck dissection. The patient received postoperative RT (60 Gy in 30 fractions). Five months after the RT, the patient was without recurrent disease.

Discussion

The failure to detect the primary tumor location in a patient with metastatic HNC poses a clinical challenge that can affect the course of treatment and disease prognosis. New recommendations were made in recent guidelines but were not applied in the case presented above, which further illustrates their importance in the diagnostic protocol, choice of treatment, and better overall and disease-free survival.

After clinical examination and diagnostic imaging, FNA biopsy is crucial in assessing neck nodal mass in SCCUP. The American Joint Committee on Cancer (AJCC) [3] recommended adding HPV staining to the diagnostic workup. HPV-specific marker p16 positive immunohistochemical staining would indicate a potential oropharyngeal primary tumor (palatine tonsil and base of the tongue). Lymph node metastases in SCCUP were positive for HVP in 7.8% to 30% of patients [6, 9]. In Serbia, oropharyngeal carcinoma was positive for p16 HPV in 45% [10, 11]. A positive p16 result should at least be followed by HPV-specific testing (*in situ* hybridization or PCR), especially in cases where no non-keratinizing histology or lymph nodes are not found in II and/or III region.

PET/CT is recommended in all patients where conventional imaging failed to identify the tumor's primary site. PET/CT has high sensitivity (up to 88.3%) and negative predictive value (from 68.9% to 93%), which makes it an excellent complementary diagnostic tool [7, 12, 13]. Diagnostic protocols that use preoperative PET/CT preceding EGD with directed biopsies resulted in the detection of the primary lesion in over 90% of patients [12, 13]. Results of both HPV staining and PET/CT would probably be indicative of the location of the primary tumor in case presentation but were not part of the performed diagnostics in the case presented.

National Comprehensive Cancer Network (NCCN) made recommendations for endoscopy (nasopharyngoscopy, inspection, and palpation of the oral cavity and oropharynx, laryngoscopy, esophagostomy, bronchoscopy) with tonsillectomy and directed biopsies based on the levels of the neck involved under general anesthesia [14]. Oropharyngeal sites, especially the tonsils and tongue's base, are the most common site for primary occult tumors. Tonsillectomy increased the likelihood of identifying a primary by 30%, compared to deep tonsil biopsies, where the identification rate was only 3% [15]. Bilateral tonsillectomy is preferred to unilateral due to a possible bilateral and contralateral tumor location in 15% of the cases with tonsillar malignancies [16]. Recommendation on lingual tonsillectomy is still not firmly established. With advances in operative techniques that include transoral laser microsurgery and transoral robotic surgery, lingual tonsillectomy provided a tumor detection rate of 56% in patients with SCCUP [8]. Bilateral tonsillectomy should always be performed in cases of SSCUP, while in the presented case, only blind biopsies were done in the absence of the evident tumor site. In this case, the pharyngolaryngeal tumor was considered a secondary primary, but we cannot exclude the possibility of the contralateral recurrent disease if the tonsils were positive for occult carcinoma.

Further treatment in patients with unknown primary carcinoma with neck metastases involves neck dissection followed by postoperative RT or consideration of chemo-RT [1, 15]. Multiple retrospective studies had inconsistent results regarding RT field size. Some reports reported that patients who underwent bilateral RT did not have significantly better overall survival and regional recurrence compared to patients treated with unilateral RT to the neck and mucosal surfaces. On the other hand, some studies favor bilateral nodal and mucosal irradiation [17, 18]. The NCCN recommends chemo-RT in N2/N3 disease cases with the extracapsular extension [14], although it should be noted that no randomized trials demonstrate the superiority of this treatment over RT alone. Due to the low incidence of the disease and the lack of high-quality evidence, clear clinical management protocols are not available.

Conclusion

Substantial advances in diagnostics and operative techniques have increased the likelihood of primary tumor identification and regional and systemic spread of the disease. If a CT or magnetic resonance imaging does not identify a primary site, the PET/CT scans should be performed before surgical endoscopy and biopsies. In cases of SCCUP, bilateral tonsillectomy with lingual tonsillectomy is indicated during EGD. Although high-quality evidence of treatment protocols is lacking, patients with more advanced stages of the regional disease require combined treatment in the form of neck dissection followed by concomitant radiation with or without chemotherapy.

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CASE REPORT

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Primary percutaneous coronary intervention in a patient with anomalous origin of the left coronary artery from the opposite sinus of Valsalva and left main coronary artery occlusion

Primarna perkutana koronarna intervencija kod bolesnika sa anomalnim ishodištem leve koronarne arterije iz suprotnog sinusa Valsalva-e i okluzijom glavnog stabla leve koronarne arterije

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Abstract

Introduction. Congenital coronary anomalies are detected in about 5% of all performed coronarographies. Coronary artery (CA) anomalies (CAA), considered to be of great risk, are the ones where the CA arises from the opposite sinus (anomalous origination of CA from opposite sinus, ACAOS) of Valsalva. These anomalies are detected in about 1% of cases. This report shows a unique case of a patient with anterior wall ST-elevation myocardial infarction (STEMI) caused by left main CA (LMCA) occlusion, which arose from the right coronary cusp and had an interarterial course, successfully treated with primary percutaneous coronary intervention (PCI). **Case report.** A 46-year-old male patient was admitted to the hospital due to STEMI of the anterior region. On admission, the patient was hypertensive (150/100 mmHg) in sinus rhythm (heart rate 70/min), Killip I. After the initial examination and admitting dual antiplatelet therapy, the patient underwent urgent coronarography. Coronarography was performed using the transradial approach. The right CA had no significant stenosis and was easily cannulated, whereas the left CA could not be cannulated at the usual position. Attempts to cannulate the left CA with multiple catheters of various curves were unsuccessful. The conclusion was that there was a CA anomaly, and the cannulation of the anomalous left CA, which arose from the opposite (right) coronary cusp (anomalous aortic origin of the left CA, AAOLCA), was successfully performed with a Multipurpose catheter. Moreover, the LMCA was occluded in the distal segment. Two drug-eluting stents (DES) were implanted, but the patient developed the no-reflow phenomenon and cardiogenic shock. After the patient was stabilized, computed tomography (CT) coronarography was performed, and AAOLCA with an interarterial course was registered. During the follow-up period, single photon emission computed tomography (SPECT) was performed, and in the staged procedure, a stent was implanted into the proximal circumflex artery using the T and protrusion (TAP) technique. **Conclusion.** Patients with STEMI and the anomalies of CAs are very rare. As such, these patients represent a great challenge for revascularization. Possessing the knowledge of anatomic varieties is paramount when it comes to these patients to treat them adequately with primary PCI.

Key words:
computed tomography angiography; coronary angiography; coronary occlusion; coronary vessel anomalies; percutaneous coronary intervention; sinus of valsalva; st elevation myocardial infarction.

Apstrakt

Uvod. Kongenitalne anomalije koronarnih arterija (KA) otkrivaju se kod oko 5% svih izvedenih koronarografija. Veoma rizičnim se smatraju anomalije kod kojih KA potiču iz suprotnog sinusa (*anomalous origination of coronary artery from opposite sinus,* ACAOS) Valsalva-e i registruju se kod oko 1% slučajeva. Prikazan je jedinstven slučaj bolesnika sa infarktom prednjeg zida miokarda sa ST elevacijom (*ST-elevation myocardial infarction*, STEMI) izazvanog okluzijom glavnog stabla leve koronarne arterije (LKA) porekla iz desnog koronarnog kuspisa sa interarterijskim pravcem pružanja, koji je uspešno lečen primarnom perkutanom koronarnom intervencijom (PKI). **Prikaz bolesnika.** Bolesnik, starosti 46 godina, primljen je kao hitan slučaj zbog kliničkih i elektrokardiografskih znakova za STEMI anteriorne regije**.** Po prijemu je bio hipertenzivan (150/100 mmHg), u sinusom ritmu

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(frekvencija srca 70/min), Killip I. Posle prvog pregleda i uvođenja dvojne antiagregacione terapije bolesnik je podvrgnut urgentnoj koronarografiji. Koronarografija je urađena transradijalnim pristupom. Desna KA koja je bila bez značajnih suženja je lako kanulisana, dok leva koronarna arterija nije mogla da se kanuliše. Pokušano je sa više katetera različitih krivina, ali bez uspeha. Zaključeno je da se radilo o koronarnoj anomaliji i tek sa *Multipurpose* vodič-kateterom uspešno je kanulisana leva KA čije je ishodište bilo iz desnog koronarnog kuspisa (*anomalous aortic origin of the left coronary artery,* AAOLCA). Arterija je bila i okludirana u svom distalnom segmentu. Urađena je implantacija dva stenta obložena lekom, ali je kod bolesnika došlo do zastoja koronarnog protoka i kardiogenog šoka. Nakon stabilizacije stanja bolesnika, izvedena je koronarografija uz pomoć kompjuterizovane tomografije kojom je potvrđen AAOLCA, a registrovano je i da postoji potencijalno rizični interarterijski pravac pružanja leve KA. Nakon pregleda izvršenog tehnikom kompjuterizovane tomografije sa jednom fotonskom emisijom (*single photon emission computed tomography*, SPECT), tokom perioda praćenja, u sledećoj etapi je urađena implantacija stenta u proksimalnu cirkumfleksnu arteriju uz pomoć *T and protrusion* (TAP) tehnike. **Zaključak.** Bolesnici sa STEMI i anomalijama KA se relativno retko sreću i predstavljaju izazov za revaskularizaciju, te je poznavanje anatomskih varijeteta neophodno kako bi ovi bolesnici mogli biti adekvatno tretirani primarnom PKI.

Ključne reči:
angiografija, tomografska, kompjuterizovana; angiografija koronarnih arterija; koronarna okluzija; koronarni krvni sud, anomalije; perkutana koronarna intervencija; sinus valsalvae; infarkt miokarda sa st elevacijom.

Introduction

Congenital coronary artery (CA) anomalies (CAA) are detected in around 5% of all performed coronarographies [1]. In the literature, there are 66 different anomalies described, while the ones considered of great risk are anomalous CAs from the opposite sinus (ACAOS) of Valsalva. These anomalies are detected in around 1% of cases. Anomalous aortic origin of the right CA (AAORCA) arising from the left coronary cusp is detected more often, whereas the anomalous aortic origin of the left CA (AAOLCA) arising from the right coronary cusp is detected in 0.15% of the cases [1, 2]. With both CAA, there are several different courses, such as prepulmonic, retroaortic, subpulmonic (septal), or interarterial courses, where the CA is placed between the aorta and the pulmonary trunk. The interarterial course is the only course that can cause sudden cardiac death and, therefore, is considered malignant [3]. ST-elevation myocardial infarction (STEMI) is rarely detected in patients with CAA and is demanding to treat. The very identification and cannulation of the culprit artery with anomalous origin can be quite challenging.

Herein we presented a unique case of a patient with anterolateral wall STEMI, caused by an occlusion of the left CA arising from the right coronary cusp with interarterial course, successfully treated with the primary percutaneous coronary intervention (PCI).

Case report

A 46-year-old patient was admitted to the hospital due to anterior wall STEMI (Figure 1). Chest pain started 2 hrs prior to admission to the hospital. The patient had no prior medical history, and the only risk factor for CA disease was smoking. On admission, the patient was hypertensive (150/100 mmHg) in sinus rhythm (heart rate 70/min), Killip I. After the initial examination, dual antiplatelet therapy was introduced, and the patient was transferred to the catheterization unit for urgent coronarography.

Coronarography was performed using a transradial approach. The right CA was easily cannulated with diagnostic catheter Tiger (Terumo, Japan), and it had no significant stenosis, whereas the left CA could not be cannulated in the left coronary cusp. Cannulation of the left CA was attempted with multiple catheters, such as EBU of various curves, JL 4.0, and Mach 1™ Amplatz, but these attempts were unsuccessful.

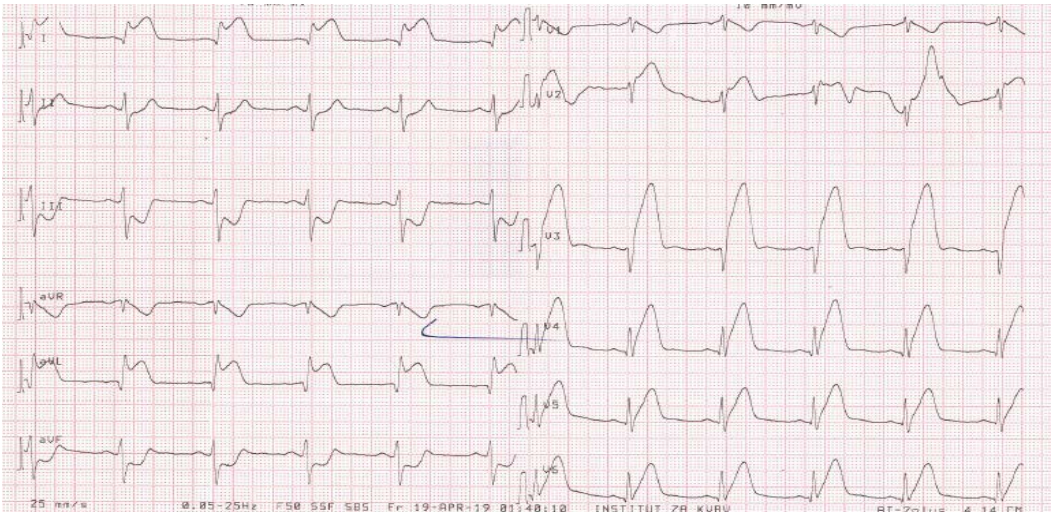

Fig. 1 – Electrocardiographic (ECG) finding of ST-elevation myocardial infarction on admission.

The operator concluded there was an existing CAA, and the cannulation of the AAOLCA from the right coronary cusp, which was occluded in its distal segment, was successfully cannulated with a Mach 1™ Multipurpose catheter (Boston Scientific, USA) (Figure 2A). After passing the guidewire through the occlusion, the antegrade flow was achieved, followed by ventricular fibrillation and prolonged cardiopulmonary resuscitation. Finally, the restoration of spontaneous circulation (ROSC) was established. Due to the progression of heart failure to the state of cardiogenic shock, the patient was intubated and put on mechanical ventilation, and vasopressor support was introduced in the treatment.

Because of the loosing of the position of the guiding catheter and clinical instability, crossover to the right femoral access was performed. This time, the left CA was cannulated with a Mach 1™ JR 4.0 guiding catheter (Boston Scientific, USA). After passing the guidewire, thrombus aspiration was performed due to the high thrombus burden. Subsequently, predilatation was performed, and after that, TIMI 2 flow was established. A trifurcation lesion of the left main CA (LMCA) was detected. The plaque was propagating from distal LMCA to the proximal left anterior descending artery (LAD) and circumflex (Cx), while the gracile Ramus had no significant lesion at the ostia (Figure 2B). Two drug-eluting stents (DES) were implanted, one DES 3.5 × 23 mm (Xience Xpedition, Abbott, USA) from the LMCA to the LAD. The second DES 3.5 × 15 mm (Xience Xpedition, Abbott, USA) was implanted in the LMCA, with a short overlap with the previously implanted stent. Furthermore, a proximal optimization technique (POT) with a semi-compliant balloon, 4.0 × 12 mm (Sprinter, Medtronic, USA), with high-pressure inflation, was performed. After POT, a no-reflow phenomenon developed (Figure 2C), and GP IIb/IIIa antagonist (tirofiban) was admitted intracoronary. Furthermore, an intra-aortic balloon pump (IABP) was implanted (Figure 2D), and the patient was transferred to the coronary care unit (CCU). After 48 hrs in the CCU, the patient was clinically stable, and both vasopressor support and IABP were removed. The patient was extubated after 72 hrs. Echocardiography registered an ejection fraction of 40% with akinesia of the apex and all apical segments of the left ventricle and hypokinesia of the medial anterolateral and inferolateral wall.

On the seventh day of hospitalization, the patient was stabilized and transferred to the ward. Computed tomography (CT) coronarography was performed for detailed analyses of the CAA. CT registered a slit-like AAOLCA from the right

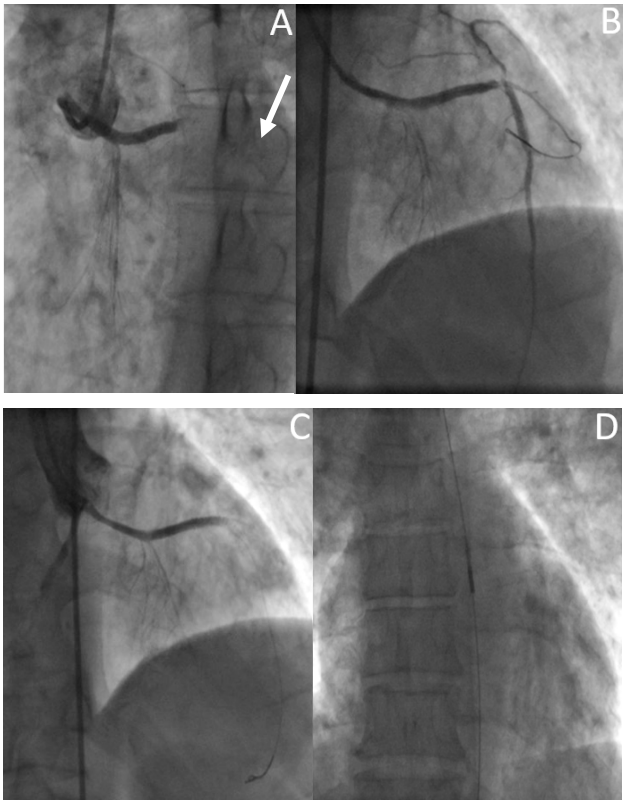

Fig. 2 – A) The arrow points to the occlusion site of the distal segment AAOLCA, LAO 46, CAU 3; B) Anterograde flow is established, and a trifurcation lesion is detected, RAO 2, CRA 26; C) No-reflow at the end of the procedure, RAO 14, CRA 26; D) Positioning of IABP, AP 0. AAOLCA – anomalous aortic origin of the left coronary artery; LAO – left anterior oblique view; CAU – caudal view; RAO – right anterior oblique view; CRA – cranial view; IABP – intra-aortic balloon pump; AP – anteroposterior view.

coronary cusp with the interarterial course (placed between the aorta and pulmonary trunk). Furthermore, the length of the LMCA was 50 mm. Interestingly, the artery at the point of 7.2 mm from the orifice at the aorta enters the heart muscle and passes through up to trifurcation in all its lengths. The stents were patent, and at the Cx ostia, a significant lesion was registered (Figure 3). On the fourteenth day of the hospitalization, the patient was discharged in good general condition. During the six-month follow-up period, the patient had symptoms of stable angina. Single photon emission computed tomography (SPECT) was performed, and it showed significant ischemia in the irrigational area of Cx.

Ten months after the STEMI, repeat coronorography was performed and, this time, a left Amplatz 1 guiding catheter was used, and successful cannulation of LCA and RCA was achieved. Coronography registered significant stenosis of the ostial Cx, and the previously implanted stents were patent. As a result, DES 2.5 × 15 mm (Xience Pro, Abbott, USA) was implanted on the ostium of Cx with the T and protrusion (TAP) technique (Figure 4).

Discussion

The report presented a case of a STEMI patient with AAOLCA from the right coronary cusp with an interarterial course. Besides, the patient had LMCA occlusion in the distal segment. To the best of our knowledge, this is a unique case in the literature.

The usage of noninvasive technologies, such as CT and MRI, enables detecting and registering coronary anomalies

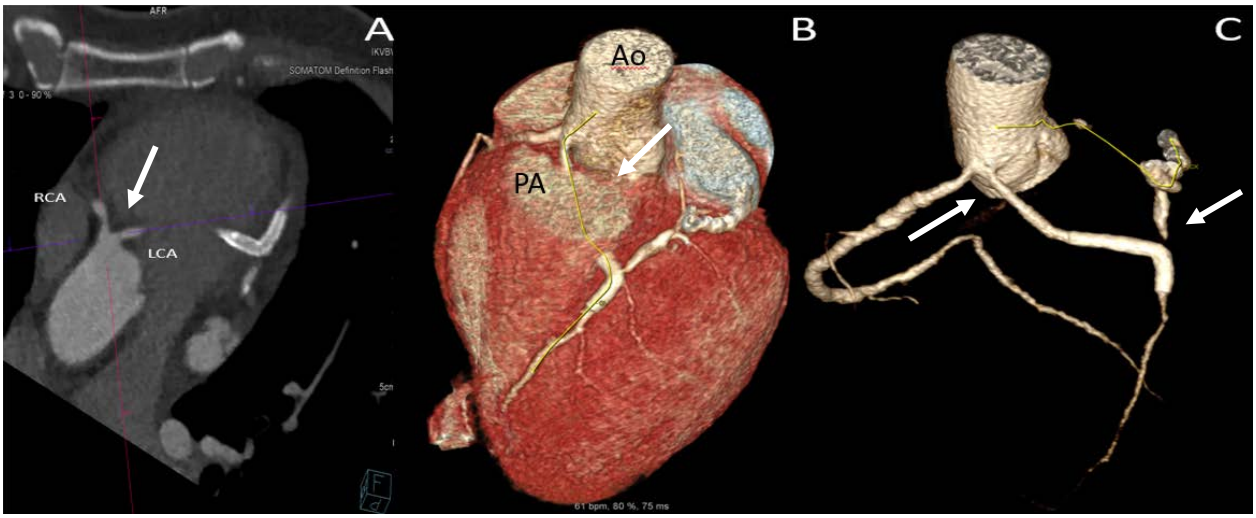

Fig. 3 – A) The arrow points to the anomalous origin of LCA from the opposite sinus of Valsalva; B) 3D reconstruction registers the interarterial course of AAOLCA, the arrow points to the part of the artery which is inside of the muscle (Ao – aorta, PA – pulmonary artery); C) The left arrow points to the slit-like orifice of AAOLCA, the right arrow points to significant stenosis of ostial circumflex (Cx). LCA – left coronary artery; AAOLCA – anomalous aortic origin of the left coronary artery.

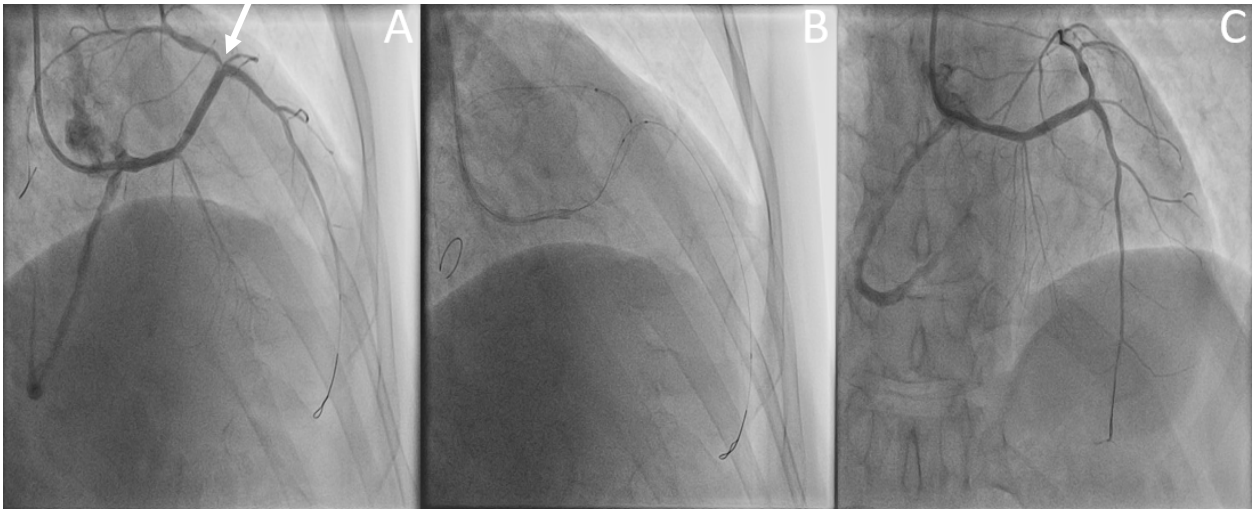

Fig. 4 – A) The arrow points to a lesion at the circumflex (Cx) ostia RAO 46, CRA 30; B) T and protrusion (TAP) technique, DES 2.5 × 15 mm in Cx and NC balloon, 5 × 12 mm in LMCA, LAD 3, RAO 46, CRA 30; C) Final result, LAO 7, CRA 25. RAO – right anterior oblique view; CRA – cranial view; DES – drug-eluting stent; NC – non-compliant; LMCA – left main coronary artery; LAD – left anterior descending artery; LAO – left anterior oblique view.

more frequently [3, 4]. The detection of CAA deserves special attention. Even though most of these anomalies are benign, CA arising from the opposite sinus of Valsalva with interarterial course presents a risk of sudden cardiac death. It can be caused by many various factors, such as a slit-like orifice or tangential passage of origin, or it might result from extreme physical activity, which can lead to the compression of the anomalous coronary artery with an interarterial course. Extreme physical activity can result in increased blood flow through the aorta and pulmonary artery leading to compression, which causes ischemia. It is crucial to emphasize that these anomalies present a great risk for the younger population, which is exposed to extreme physical activity. That mostly refers to sportsmen, athletes, and military recruits. Sometimes, the first manifestation can be sudden cardiac death (SCD); however, very often, there are symptoms present during physical activity, such as angina-like symptoms, arrhythmia, presyncope, and syncope [5, 6].

The literature provides us with a different frequency of atherosclerotic disease in anomalous CAs compared to normal. Recent studies indicate a slightly higher incidence of CA disease (CAD) in anomalous CAs than what was shown in earlier studies which indicated an equal incidence of CAD [7]. However, STEMI patients with coronary anomalies are quite rare. According to the research conducted by Marchesini et al. [8], only 5 out of 1,015 STEMI patients (0.4%) had an anomaly of the CA at the same time.

Performing primary PCI in STEMI patients and CAA is challenging for every operator, mostly because of the cannulation problems of the anomalous culprit artery. However, a problem might arise during the procedure in terms of balloon and stent deliverability in the culprit lesion area. In this case report, the cannulation of the LCA presented a problem, considering that it could not be detected in the left coronary cusp. After exchanging multiple catheters, the operator decided to use a Multipurpose catheter and search for the left CA in the right coronary and posterior cusp. Luckily, in this case, only the cannulation was challenging, considering the anomalous origin. In both procedures, there was no problem with the device deliverability.

The occlusion of the LMCA is a disastrous event, and most patients have a fatal outcome on the way to the hospital. De Luca et al. [9] registered an incidence of 0.8% in patients with myocardial infarction and LMCA occlusion. Their study showed high intrahospital mortality, which was 58% of all patients and 80% of patients who developed cardiogenic shock or had no-reflow at the end of the procedure. Certain factors indicated that they contribute to the higher survival rate of these patients, such as dominant RCA, the existence of hetero-collateral circulation, and fast revascularization [10–12]. In our case, RCA was dominant, and hetero-collateral showed the very periphery of LAD. However, the existence of hetero-collateral circulation did not imply the anomalous origin of LCA. The operator's experience in assuming the presence of coronary anomaly and the adequate selection of catheter allowed successful revascularization of LMCA and proximal LAD.

Despite the loss of around 15 minutes until the cannulation of anomalous LCA, guidewire passage, and establishing anterograde flow through the infarction artery, cardiogenic shock as well as no-reflow at the end of the procedure developed. No-reflow developed as a consequence of a high thrombus burden and aggressive post-dilatation during POT, which led to distal embolization with thrombus masses and debris from the lesion, causing a microvascular obstruction. Treatment of no-reflow was challenging because nitroglycerin, adenosine, or intracoronary adrenalin could not be given due to hemodynamic and rhythmic instability. The decision to administer GP IIb/IIIa antagonist (tirofiban) with implantation of mechanical circulatory support with IABP and aggressive treatment of cardiogenic shock with the support of invasive mechanical ventilation led to fast stabilization. It is important to emphasize that the veno-arterial extracorporeal membrane oxygenation (VA-ECMO) was considered. However, due to the no-reflow and admission of GP IIb/IIIa antagonist, there was a high risk of bleeding; thus, it was decided to wait and monitor the patient's status. As satisfactory hemodynamical stabilization was achieved promptly during the treatment at the CCU, the implantation was no longer needed.

Since 3D reconstruction is not possible, angiography has a limited sensitivity when it comes to adequate diagnostics of anomalies of CAs. Therefore, to diagnose the type of anomalies, their course, and their placement in relation to great vessels, it is necessary to use multi-slice computed tomography or magnetic resonance imaging. Applying these methods prior to coronarography can facilitate the cannulation of the anomalous CA, and this option is manageable in elective and stable patients [13].

A surgical approach to AAOLCA and AAORCA anomalies is considered in patients under 35 years of age, who were exposed to high physical efforts, and who had experienced some of the symptoms which led to the diagnosis of this type of anomaly. When it comes to older patients, the type of treatment should be carefully considered, the need for surgical intervention in particular. Whether these patients are asymptomatic is also of great importance. Tests for determining ischemia are advised in these patients [14]. Surgery treatment was not taken into consideration for the patient presented in this case report since the patient did not have any symptoms before the coronary incident and was not exposed to great physical efforts. SPECT showed ischemia only in the Cx area, which was sub-occluded at the origin and was revascularized by stent implantation.

Conclusion

STEMI patients with CAA are rare and represent a challenge for revascularization; thus, knowing the anatomical varieties is essential to treating these patients with PCI.

Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

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The first case report of a solitary metastasis of the transitional cell carcinoma of the ovary to the spleen

Prvi slučaj izolovane metastaze primarnog karcinoma prelaznih ćelija jajnika u slezinu

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Abstract

Background. Primary transitional cell carcinoma (TCC) of the ovary is characterized by the presence of papillary projections of malignant transitional epithelial cells or their aggregates in the fibrous stroma. This type of tumor represents nearly 1% of all ovarian surface epithelium carcinomas. We presented the first report of a solitary splenic metastasis of primary ovarian TCC. **Case report.** A 60-year-old female patient was admitted because of an asymptomatic splenic tumor in December 2018. Two years prior, she underwent a total abdominal hysterectomy, bilateral adnexectomy, and infracolic omentectomy for the primary TCC of the ovary. Control abdominal ultrasonography, computed tomography, and magnetic resonance imaging performed two years after primary surgery showed a splenic tumor. An open splenectomy was performed, with the intraoperative finding of a hilar splenic tumor and the absence of other pathological lesions in the abdomen. Frozen section analysis showed a TCC metastasis, which was subsequently confirmed by definitive histopathological examination. During the one-year follow-up, there was no relapse of the disease. **Conclusion.** This is the first report of a solitary splenic metastasis of primary ovarian TCC based on the literature review. This case may serve as an example of the diagnostic and therapeutical role of splenectomy in isolated splenic metastases of ovarian cancer.

Key words:
carcinoma, transitional cell; diagnosis; histological techniques; neoplasm metastasis; ovarian neoplasms; spleen.

Apstrakt

Uvod. Primarni karcinom prelaznih ćelija (KPĆ) jajnika karakteriše prisustvo papilarnih projekcija malignih ćelija prelaznog epitela ili njihovih agregata u fibroznoj stromi. Ovaj tip tumora obuhvata oko 1% svih karcinoma površinskog epitela jajnika. Prikazali smo prvi slučaj solitarne metastaze primarnog ovarijalnog KPĆ u slezinu. **Prikaz bolesnika.** Bolesnica stara 60 godina primljena je u decembru 2018. godine, zbog asimptomatskog tumora slezine. Dve godine ranije joj je zbog primarnog KPĆ jajnika urađena totalna abdominalna histerektomija, bilateralna adneksektomija i infrakolična omentektomija. Kontrolna ultrasonografija abdomena, kompjuterizovana tomografija i magnetna rezonanca, sprovedene dve godine nakon operacije, pokazale su tumor slezine. Urađena je otvorena splenektomija, a intraoperativni nalaz je pokazao tumor hilusa slezine, bez drugih patoloških lezija u abdomenu. Patohistološka analiza je pokazala metastazu KPĆ, što je potvrđeno naknadnom definitivnom patohistološkom analizom. U toku jednogodišnjeg praćenja nije bilo relapsa bolesti. **Zaključak.** Prema literaturnim podacima ovo je prvi prikazani slučaj solitarne metastaze primarnog KPĆ jajnika u slezinu, koji može predstavljati primer dijagnostičke i terapijske uloge splenektomije kod izolovanih metastaza karcinoma jajnika u slezinu.

Ključne reči:
karcinom prelaznih ćelija; dijagnoza; histološke tehnike; neoplazme, metastaze; jajnik, neoplazme; slezina.

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Introduction

Ovarian transitional cell tumors may present as transitional cell carcinoma (TCC), as well as benign, borderline, or malignant Brenner tumors, in total accounting for nearly 2% of all ovarian tumors [1]. It is considered that Brenner tumors arise from the surface epithelium and stroma through the process of transitional cell metaplasia [2] and that around 1% of all Brenner tumors are malignant [3]. Primary TCC of the female genital tract is described in the ovary, vagina, uterine cervix, endometrium, and Fallopian tubes [4]. Primary ovarian TCC was first described by Austin and Norris in 1987 [5]. It represents 1% of all ovarian surface epithelium carcinomas [6]. The lack of urothelial markers suggests a Mullerian origin of TCC, therefore distinguishing it from urothelial cancer [7]. TCC is characterized by the lack of the Brenner component [8, 9] and the lack of stromal calcification [10]. On the other hand, TCC shows malignant transitional type cells in papillary proliferations or aggregates in the fibrous stroma [6]. Silva et al. [11] showed that focal or diffuse ovarian TCC components presented in 88 of 934 ovarian cancer cases. Primary ovarian TCC has a better prognosis compared with other ovarian carcinomas due to a higher degree of chemosensitivity [6, 7, 10].

We present the first case of a solitary splenic metastasis of primary ovarian TCC based on the histopathological examination and the medical history of the patient.

Case report

In December 2018, a 60-year-old female patient was admitted for elective splenectomy to treat an asymptomatic splenic tumor. In 2016, she underwent a total abdominal hysterectomy, as well as bilateral adnexectomy and infracolic omentectomy for a massive pelvic tumor. The initial imaging finding [abdominal computed tomography (CT) scan interpretation] did not show any evidence of other intraabdominal pathological lesions, confirmed by the operative report from primary surgery (which was not performed in our institution). Multiple biopsies from the visceral peritoneum (mesentery) as well as the parietal peritoneum (central, anterolateral, and pelvic peritoneum) were taken. Histopathology of the pelvic tumor showed a primary ovarian TCC with infiltrative growth and partial necrosis. It also showed papillary projections of pleomorphic epithelial cells expressing multiple mitoses and acidophilic cytoplasm. Immunohistochemistry stain showed CK7 positivity and CK20 negativity. The tumor stage was determined as pT1c, histologic grade 2–3, and nuclear grade 3. The peritoneal biopsies were all negative. Afterward, she underwent six cycles of chemotherapy (paclitaxel and carboplatin). Other medical history was unremarkable.

On admission, the patient did not report any symptoms, and the physical finding was normal (besides the scar from the previous laparotomy). A preoperative abdominal ultrasonography exam (performed during the oncological follow-up) showed a splenic mass consisting of multiple focal lesions (48 × 32 mm; vertical and transverse diameter, respectively). An abdominal CT scan showed an interpolar splenic mass (42 × 42 × 36 mm; vertical, laterolateral, and anteroposterior diameter, respectively) (Figure 1). Magnetic resonance imaging (MRI) showed a 10.0 × 5.5 cm sized spleen (vertical and laterolateral diameter, respectively) with a tumor located on the superior aspect of the splenic hilum, posteriorly from the stomach (Figure 2). The tumor size was 44 × 24 × 36 mm (vertical, laterolateral, and anteroposterior diameter, respectively). The lesion showed diffusion restriction and was hypovascular in comparison with the splenic parenchyma. The other imaging findings in the abdomen, as well as chest X-ray and head CT, were normal. The laboratory results showed that CA 125 was elevated (50.6 U/mL). Other results (complete blood count, biochemical parameters, and other tumor markers) were normal.

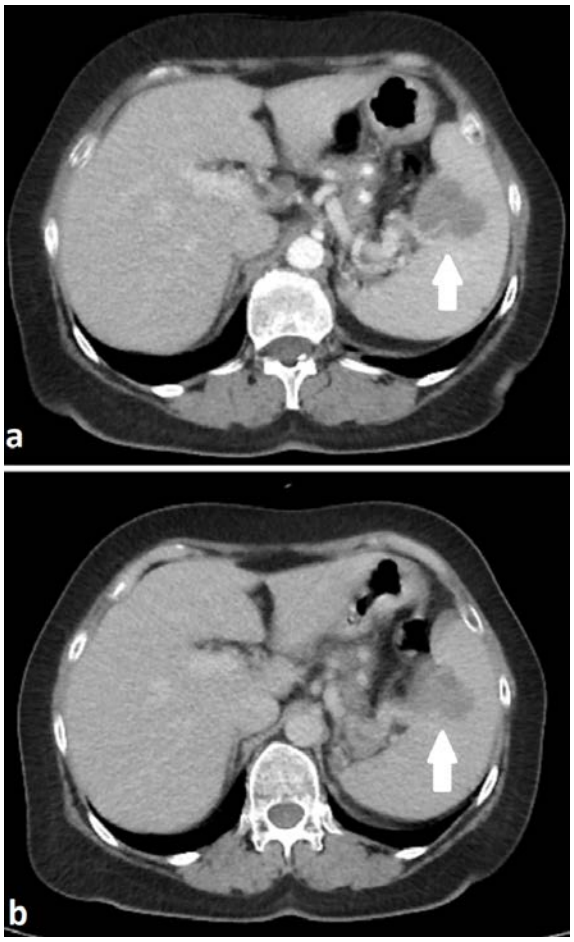

Fig. 1 – Contrast-enhanced axial computed tomography scans of the upper abdomen with the arrow indicating the splenic tumor: a – arterial phase; b – delayed phase.

After the patient was presented with the risk of potential complications of laparoscopic splenectomy being performed after the previous laparotomy, she was suggested for open splenectomy. A left subcostal laparotomy was performed, with the intraoperative finding of a splenic hilar tumor in close contact with the tail of the pancreas and the posterior gastric wall. Further exploration did not reveal any locoregional relapse of TCC, peritoneal dissemination, or metastatic disease in other organs. An open splenectomy was per-

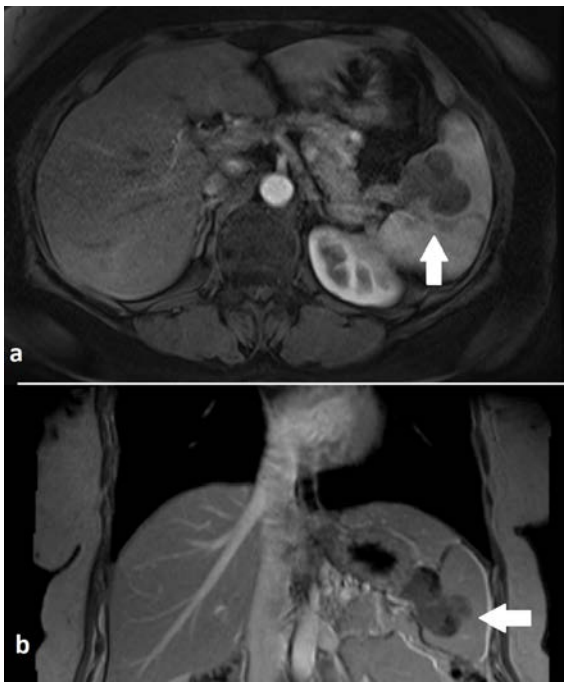

Fig. 2 – Contrast-enhanced T-1 weighted magnetic resonance imaging of the abdomen with the arrow indicating the splenic tumor: a – axial slice, arterial phase; b – coronal slice, delayed phase.

formed, and the splenic bed was drained with two surgical drains. The tumor exhibited yellowish and greenish color with a lobular structure (Figure 3). Frozen section analysis was suggestive of TCC metastasis. Histopathology showed malignant transitional type cells organized into papillary structures (Figure 4), multiple pathologic mitoses, CK7 positivity and CK20 negativity. This histopathological finding was seen in the hilar lymph nodes of the spleen as well. The final conclusion was that the splenic tumor represented a metastasis of the primary ovarian TCC.

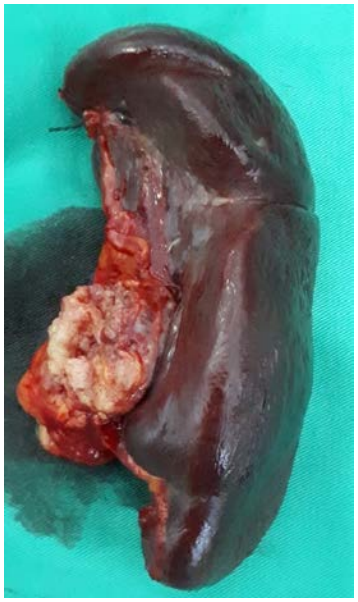

Fig. 3 – Gross examination finding of the removed spleen.

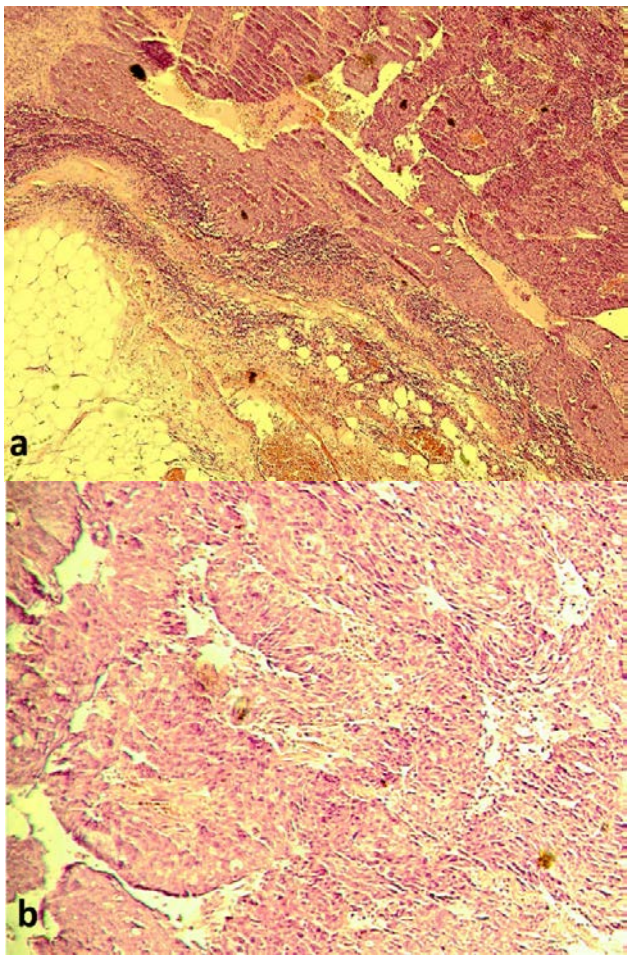

Fig. 4 – Histopathology finding (haematoxylin-eosin staining): a – ×50; b – ×100.

The recovery was uneventful, and the patient was discharged on the seventh postoperative day. Postsplenectomy antimicrobial prophylaxis was performed, including pneumococcal, meningococcal, and *Haemophilus influenzae* type b vaccinations. Postoperative oncological treatment consisted of six cycles of paclitaxel and carboplatin. A one-year follow-up (chest and abdominal CT, abdominal MRI, and CA 125 levels) did not show any recurrence of the disease.

Discussion

This report is based on a late manifestation of primary ovarian TCC in a solitary metastatic behavior to the spleen. The metastatic pathway of ovarian TCC resembles the metastases from urothelial carcinoma due to the loss of E-cadherin [10]. In a study on 302 patients [with 5.3% (16 patients) suffering from primary TCC], Kommoss et al. [7] showed that primary ovarian TCC exhibits micronodular extraovarian growth more commonly than other ovarian cancers (usually characterized by direct macronodular spreading). Owing to this, primary TCC often has a lesser preoperative extraovarian component, as well as a smaller extent of postoperative residual tissue leading to a superior 5-year

survival (57.14%) compared with non-TCC ovarian carcinomas (30.68%). In 2008, Keepanasseril et al. [12] presented a patient with right-sided cervical lymphadenopathy (levels II and III) as a solitary metastasis of right-sided primary TCC of the ovary, without metastases to the abdomen and thorax.

Metastatic tumors of the spleen are usually accompanied by malignant peritoneal dissemination [13], while solitary splenic metastases usually arise from gastrointestinal cancers [14]. A literature review by Izuishi et al. [15] in 2010 showed that 27% of all solitary splenic metastases arise from ovarian cancer. Bearing in mind that there are several papers published before the year 2000 [16], Table 1 contains short descriptions of solitary ovarian cancer metastases to the spleen published in the relevant literature as of the year 2000 (also, there are several papers published before the year 2000) [17–25]. Based on the literature review, we can conclude that this is the first reported case of a solitary splenic metastasis of primary ovarian TCC. It is considered that solitary splenic metastases are rare due to the sharp angle of splenic artery origin from the celiac trunk, the contractile washout of blood from the splenic sinusoids to the splenic vein, the scarcity of afferent lymph nodes, as well as to the inhibitory effect of the histological milieu of spleen on the growth of malignant tissue [13, 26]. Unlike the metastases to the liver parenchyma, splenic metastases are not considered stage IV malignant disease. Splenectomy is described as a part of the first-step cytoreductive surgery for ovarian cancer, as well as secondary cytoreduction – independently from the presence of splenic metastases [16]. Farias-Eisner et al. [14] hypothesized that the spleen could present as "a pharmacological and immunological sanctuary" for ovarian cancer cells.

Primary ovarian TCC is treated with optimal surgical resection and cisplatin-based chemotherapy [10, 27]. Ichigo et al. [10] showed that surgical resection with postoperative cisplatin results in superior survival. A 5-year follow-up of 88 patients showed a survival rate of 37% in the group of patients treated with surgery (as the only treatment method) and 41% in the group that underwent surgery combined with chemotherapy. They concluded that the TCC component contributed to a better prognosis, which depends on the clinical stage of the disease [10].

The patients in Table 1 had a disease-free interval from 11 months to 5 years after splenectomy. The case presented herein exhibits splenectomy as a diagnostic step (to determine the presence of metastatic disease), as well as a curative approach (with a one-year disease-free interval after surgery). This may serve as an inspiration to report solitary ovarian TCC metastases to the spleen in order to recognize the true incidence of this metastatic pattern, as well as the therapeutic benefit from splenectomy.

The importance of differentiation between primary ovarian TCC and metastatic urothelial cancer lies in the fact that the presence of malignant urothelial cells leads the diagnostic approach in the direction of searching for a primary urinary tract cancer. Badin et al. [1] presented an 83-year-old female patient who had an ovarian tumor surgery, with the histopathological finding inconclusive between primary ovarian TCC and metastatic urothelial cancer. Six years prior, she underwent transurethral resection of a bladder urothelial cancer, with subsequent intravesical administration of interferon-alpha and Bacillus-Calmette-Guerin vaccine. This anamnestic information – together with immunohistochemical positivity of the tumor to CK7 and CK20 – leads to the conclusion that this was a metastatic urothelial carcinoma. Lee et al. [28] presented two female patients with metastatic urothelial carcinomas to the ovary (from the renal pelvis and the bladder). Their literature review showed that urothelial carcinoma metastases to the ovarium are rare and that the most frequent metastases from the urinary tract to the ovarium were from clear cell renal adenocarcinoma. Ichigo et al. [10] stated that the most significant parameters in differentiating between primary ovarian TCC and urothelial cancer are positivity to CK7, CK20, uroplakin III, and Wilms tumor protein. Moreover, primary ovarian TCC exhibits broad papillae with mucin collections, while metastatic urothelial cancer forms pseudo-papillae after necrosis of the tumor cells [28].

Urothelial carcinoma[4] and malignant Brenner tumors express CK7 and CK20 positivity, while Mullerian serous tumors express only CK7 positivity [1, 9]. Ovarian TCC is unreactive with CK20 [7, 27] and uroplakin III [6, 12], while 30% of ovarian TCC are reactive with thrombomodulin. On the other hand, ovarian TCC expresses positivity for Wilms tumor protein 1 [1], vimentin, and CA 125 [2]. In benign and borderline Brenner tumors, p63 is expressed. On the other hand, its expression is absent in malignant Brenner tumors and primary ovarian TCC [10]. Cuatrecasas et al. [2] showed an increase in p16 and p53 expression as well as more frequent p53 mutations in primary ovarian TCC compared with malignant Brenner tumors. This characterizes primary ovarian TCC as a high-grade tumor. Coffman et al. [29] combined human and murine models to show the tropism of high-grade ovarian cancer cells for the ovary, therefore supporting the role of hematogenous spread of ovarian cancer [30]. Furthermore, the authors comment on the possible role of oophorectomy in preventing peritoneal metastases and ascites. Owing to this, it is interesting to consider that primary surgery reduced the risk of peritoneal metastases in our patient. On the other hand, given the fact that the circulating tumor cells (paramount in hematogenous metastatic route [31]) are present in nearly 50% of all the International Federation of Gynecology and Obstetrics stage I-II ovarian cancers [30], this supports the theory of hematogenous spread to the spleen in the patient presented herein. Despite the fact that the relevant literature does not contain data on the ovarian TCC metastasis growth rate, it is known that the survival rate for TCC patients is similar to the survival rate for advanced high-grade serous carcinoma [32].

Table 1

Solitary metastases of ovarian cancer to the spleen reported in the relevant literature from the year 2000 to present

Authors and year	Age of patient (years)	Histologic type of cancer	Grade	Stage (FIGO)	Chemotherapy after first surgery	Time after first surgery	Elevated CA 125	Relapse*
Yano et al., 2002 [17]	38	serous adenocarcinoma	-**	IIIC	-	3 years	-	-
Koh et al., 2004 [18]	29	mucinous (borderline)	-	-	-	1 year	yes	yes (2 years)
Tserkezoglou et al., 2005 [19]	53	serous cystadenocarcinoma	-	IIIB	cisplatin	27 months	yes	no (20 months)
Otrock et al., 2006 [20]	59	serous adenocarcinoma	high	IIA	carboplatin+paclitaxel	6 years	yes	no (11 months)
Izuishi et al., 2010 [15]	52	serous adenocarcinoma	-	IIC	5-FU; adriamycin; cisplatin; cyclophosphamide	20 years	no	no (5 years)
Karni et al., 2014 [21]	56	endometrioid-type	3	IA	carboplatin i paclitaxel	6 years	yes	-
Lee et al., 2014 [22]	66	serous adenocarcinoma /squamous cell carcinoma	-	-	-	48 months	yes	-
Resta et al., 2014 [23]	67	adenocarcinoma	-	-	-	10 years	yes	no (1 year)
Lv et al., 2014 [24]	53	clear cell adenocarcinoma	-	-	cisplatin+docetaxel	simultaneously	yes	-
Sorbi et al., 2015 [25]	66	tuboovarian serous carcinoma	2	IIIA	carboplatin+paclitaxel; trabectedin+doxorubicin	5 years	no	no (16 months)

*** – relapse of cancer after splenectomy (the follow-up period is given in brackets); ** – no available information.**
FIGO – International Federation of Gynecology and Obstetrics.

Conclusion

This is the first case report of solitary ovarian TCC metastasis to the spleen. Additionally, this case report can serve as an example of therapeutic splenectomy in solitary TCC splenic metastasis. The follow-up of this patient, as well as reporting other similar cases in the future, will demonstrate the effect of this metastatic pattern and splenectomy on the 5-year survival rate and the disease-free interval in primary ovarian TCC.

Conflict of interest

All authors declare no conflict of interest.

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Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

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MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**)**.** The use of colors and shading in graphs should be avoided.

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Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

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a) The title should be concise but informative, while subheadings should be avoided;

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d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

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The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

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The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

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Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

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Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

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

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Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

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

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

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

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

Tabele

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