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ORIGINAL ARTICLES / ORIGINALNI RADOVI

Louis Pasteur in his laboratory, painting by Albert Edelfelt, 1885.
Luj Paster u svojoj laboratoriji, slika Alberta Edelfelta iz 1885.

In December 2022, the world celebrates the 200th anniversary of the birth of Louis Pasteur (1822–1895), a scientist whose series of revolutionary discoveries are the foundations of modern microbiology, immunology, chemistry, medicine, etc. Among his most significant discoveries are the process of pasteurization, the proof that microorganisms are the cause of disease in humans, and the development of vaccines against fowl cholera, anthrax, pig erysipelas, and rabies. These discoveries, which fundamentally changed how we look at infectious diseases and how we fight them, have extended the human lifespan and saved millions of lives.

U decembru 2022. godine svet obeležava 200 godina od rođenja Luja Pastera (1822–1895), naučnika čiji je niz revolucionarnih otkrića u temeljima moderne mikrobiologije, imunologije, hemije, medicine i dr. Među njegovim najznačajnijim otkrićima su postupak pasterizacije, dokaz da su mikroorganizmi izazivači bolesti kod ljudi, kao i razvoj vakcina protiv pileće kolere, antraksa, svinjskog erizipela i besnila. Ova otkrića, koja su fundamentalno promenila ugao našeg posmatranja na zarazne bolesti i način borbe protiv njih, produžila su ljudski vek i spasla milione ljudskih života.

Dear Authors, Editors, Peer Reviewers and Readers of the *Vojnosanitetski pregled*,

We thank you for cooperation and support in 2022 and wish you all the best in the upcoming 2023!

Happy holidays!

Editorial staff of the *Vojnosanitetski pregled*

Dragi autori, urednici, recenzenti i čitaoci *Vojnosanitetskog pregleda*,

Uz zahvalnost na saradnji i podršci u 2022, želimo vam sve najbolje u nastupajućoj 2023. godini!

Srećna Nova godina i Božićni praznici!

Redakcija *Vojnosanitetskog pregleda*

ORIGINAL ARTICLES

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Relationship and influences of behavioral and psychological factors on metabolic control of patients with type 2 diabetes mellitus

Povezanost i uticaj bihejvioralnih i psiholoških faktora na metaboličku kontrolu obolelih od dijabetesa melitusa tipa 2

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Abstract

Background/Aim. Achieving good metabolic control, which plays a key role in reducing or preventing macrovascular and microvascular complications of diabetes mellitus (DM), requires continuous patient involvement in the self-management of DM. This continued engagement, which makes type 2 DM (T2DM) one of the most physically and emotionally demanding diseases, can become, at certain periods of life, extremely severe and lead to emotional distress (symptoms of depression and DM-related distress) and deterioration of metabolic control. The aim of this study was to examine the association and influence of behavioral and psychological factors on the metabolic control of patients with T2DM. **Methods**. The research was conducted as a descriptive-analytic cross-sectional study. The method of random sampling included 324 subjects with T2DM in the research. The values of biochemical parameters of metabolic control were measured by standard laboratory methods. Blood pressure was measured two times, and the arithmetic mean was calculated. Anthropometric measurement was performed, and body mass index (BMI) was calculated. Attitudes toward medication adherence, adherence to dietary recommendations, level of physical activity, presence of depressive symptoms, and level of DM-related distress were examined using standardized questionnaires. **Results.** The target values of metabolic control parameters were reached by 21.6% of respondents. Multivariate analysis as predictors of poor metabolic control identified obesity, non-adherence toward dietary recommendations, insulin therapy, low level of physical activity, and clinically significant DM-related distress. **Conclusion.** Routine application of the questionnaire used in this study in the initial stages or critical moments of the disease can assess patients' attitudes and knowledge about behavioral determinants of DM self-management and timely detect psychological conditions that affect them. It would be realistic to expect that such a comprehensive holistic approach would contribute to a lower incidence of complications and better metabolic control of T2DM.

Key words:
depression; diabetes mellitus, type 2; obesity; prognosis; risk assessment; psychology; surveys and questionnaires; therapeutics.

Apstrakt

Uvod/Cilj. Postizanje dobre metaboličke kontrole, koja ima ključnu ulogu u redukciji ili prevenciji makrovaskularnih i mikrovaskularnih komplikacija dijabetesa melitusa (DM), zahteva kontinuiranu angažovanost bolesnika u upravljanju njihovom bolešću. Ta kontinuirana angažovanost, koja čini DM tipa 2 (T2DM) jednom od najzahtevnijih bolesti, kako fizički tako i emocionalno, u određenim periodima života može postati preteška i dovesti do emocionalnog distresa (simptomi depresije i distres povezan sa DM) i pogoršavanja metaboličke kontrole. Cilj rada bio je da se ispitaju povezanost i uticaj bihejvioralnih i psiholoških faktora na metaboličku kontrolu bolesnika sa T2DM. **Metode.** Istraživanje je sprovedeno kao deskriptivno-analitička studija preseka metodom slučajnog uzorka. U istraživanje su bila uključena 324 bolesnika sa T2DM. Vrednosti biohemijskih parametara metaboličke kontrole merene su standardnim laboratorijskim metodama. Krvni pritisak je meren u dva vremena i računata je aritmetička sredina. Vršena su antropometrijska merenja i računat je indeks telesne mase. Standardizovanim upitnicima ispitivani su stavovi bolesnika prema medikamentoj adherentnosti, adherentnost prema dijetetskim preporukama, nivo fizičke

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aktivnosti, prisustvo simptoma depresije i nivo distresa povezanog sa DM. **Rezultati.** Ciljne vrednosti parametara metaboličke kontrole dostiglo je 21.6% ispitanika. Kao prediktori loše metaboličke kontrole, multivarijantnom analizom identifikovani su gojaznost, neadherentnost prema dijetetskim preporukama, terapija insulinom, nizak nivo fizičke aktivnosti i klinički značajan distres povezan sa DM. **Zaključak.** Rutinska primena upitnika korišćenih u ovoj studiji u inicijalnom stadijumu ili kritičnim fazama pogoršanja bolesti, omogućila bi procenu stavova bolesnika i znanja o bihejvioralnim determinantama upravljanja DM i blagovremeno otkrivanje psiholoških problema povezanih sa njima. Realno bi bilo očekivati da takav sveobuhvatni, holistički pristup može doprineti boljoj metaboličkoj kontroli bolesnika sa T2DM i nižoj incidenciji komplikacija.

Ključne reči:
depresija; dijabetes melitus, tip 2; gojaznost; prognoza; rizik, procena; psihologija; ankete i upitnici; lečenje.

Introduction

Diabetes mellitus (DM) represents a global public health crisis of pandemic proportions due to the growing number of patients, numerous complications that lead to disability and premature mortality, and the high cost of treatment and prevention [1, 2]. Data from International Diabetes Federation indicate that 463 million people worldwide, or 9.2% of adults aged 20–79, had diabetes in 2019 [2, 3]. Globally, about 90% of the total number of people with DM suffer from type 2 DM (T2DM) [3].

According to the data of the Institute of Public Health of Serbia, 770,000 people, or 12% of adults, suffered from DM in Serbia in 2019. This rate ranks Serbia third in Europe in the prevalence of DM [2].

Metabolic control is one of the key outcomes of DM management and involves maintaining blood glucose level, arterial blood pressure (BP), and lipid status within limits as close to normal as possible [4]. The American Diabetes Association (ADA) states the values of the following parameters as indicative of proper metabolic control: glycosylated hemoglobin (HbA1c $\leq$ 7%), low-density lipoproteins (LDL) $\leq$ 2.6 mmol/L, high-density lipoproteins (HDL) $\geq$ 1.15 mmol/L, triglycerides (TG) $\leq$ 1.7 mmol/L, and BP $\leq$ 140/90 mmHg [5, 6]. Unregulated BP and dyslipidemia are associated with insulin resistance and a lower likelihood of optimal blood glucose control, which increases the risk of macrovascular and microvascular complications of DM [7]. Despite that, in data for 2019, ADA stated that only 33–49% of patients reach target values of some of the parameters of metabolic control while reaching the target values of all the parameters is rare and amounts to 14% [8].

Achieving good metabolic control, which plays a key role in reducing or preventing complications of DM, requires continuous patient involvement in self-management of DM [9], where 90–95% of decisions about their disease are made by person independently [10]. This need for continued engagement makes T2DM one of the most demanding chronic diseases, physically and emotionally [11]. Therefore, behavioral requirements of self-management (medication adherence, physical activity, body mass control, and adherence to dietary recommendations) can, in certain periods of life, become too difficult for patients and result in poor metabolic control [12]. Perception of inability to meet the behavioral requirements of self-management of DM can result in the manifestation of emotional distress (symptoms of depression and DM-related distress), which may result in worsening of metabolic control [12, 13].

Despite the importance of the problem and attitudes about the need for a holistic and multidisciplinary approach to patients with T2DM, studies that comprehensively consider metabolic control are limited. Therefore, the aim of this study was to examine the association and influence of behavioral and psychological factors on the metabolic control of patients with T2DM.

Methods

Selection method, size, and construction of sample

The research was conducted as a descriptive-analytical cross-sectional study. The research population considered 4,620 patients with T2DM from the electronic register of the Diabetes Dispensary of the Health Center in Zaječar, Serbia. Statistical software G*Power 3.0.1. was used to estimate the required sample size for multivariate logistic regression analyses. By entering the assumed moderate effect size of r = 0.2 for a study strength of 95% and the error level α = 0.05 and potential 10 predictors, the minimum required sample size of n = 324 subjects was obtained. To reduce selection bias, respondents were determined by random sampling method, based on scheduling examination by the Integrated Health Information System performed by their chosen physicians. Thus, differences between outpatients and inpatients were avoided, and generalization to the entire sample population was provided. Exclusion criteria for all the study participants were as follows: disease duration of less than one year, subjects only on dietary therapy, and subjects with comorbidities that interfere with understanding and self-completion of the questionnaire.

The research was approved by the Ethics Committee of the Zaječar Health Center (decision number 1899/3 from April 6, 2017) and was conducted in accordance with the ethical standards specified in the Declaration of Helsinki (1964) and subsequent amendments to the declaration. After the approval of the Ethics Committee, the research was conducted from September 2018 to March 2019 at the Diabetes Dispensary of the Health Center in Zaječar.

Research implementation and methodology

Biochemical parameters of metabolic control were measured by a fully automated high-performance chromatographic test. Data on complications and comorbidities were taken from the electronic medical records of the subjects' BP. Values were obtained by measuring with a conventional

mercury sphygmomanometer in a sitting position on two occasions, after which the mean value was calculated. Body mass and body height values of the subjects were obtained by anthropometric measurements, after which body mass index (BMI) was calculated. Following ADA guidelines, the classification of the degree of nutrition was performed: BMI < 18.5 kg/m^2 – malnutrition; BMI = 18.5–24.9 kg/m^2 – normal body mass; BMI = 25–29.9 kg/m^2 – overweight; BMI ≥ 30 kg/m^2 – obesity [14].

As research tools, standardized questionnaires were used in order to reduce information errors. After the authors obtained permission to use the questionnaires that have not been used in our area so far, they were translated according to internationally recognized methodology with cultural adaptation.

Medication adherence

Medication adherence was assessed by the Questionnaire on Attitudes Toward Medication Adherence. Score 1–3 classifies subjects into a group with negative attitudes toward medication adherence, while a score > 3 indicates positive attitudes [15]. Research using this questionnaire finds its high negative predictive value (76.5–82.9%), i.e., that 76.5–82.9% of respondents with negative attitudes toward medication adherence are nonadherent toward medication therapy [15]. The assessment of the reliability of the questionnaire was based on an acceptable internal consistency (Cronbach α = 0.74).

Adherence to dietary recommendations

Adherence to dietary recommendations was assessed by the Questionnaire on the Perception of Adherence to Dietary Recommendations (PDAQ). The questionnaire consists of 9 questions designed to cover all guidelines of dietary recommendations for patients with T2DM. The total score is obtained by summing the answers to all questions [16]. In the studies in which the questionnaire was used, the respondents were classified into the group of adherents if they ate healthy for at least 4 days a week [17]. Accordingly, in this study, the point of intersection of the scale as a whole was set at 37, with a score of 1–3 indicating non-adherence, while a score > 37 indicated adherence according to dietary recommendations. The questionnaire showed acceptable internal consistency (Cronbach α = 0.78).

Level of physical activity

The level of physical activity was assessed by the Physical Activity subscale of the Personal Diabetes Questionnaire (PDQ). Physical activity was assessed with two questions: 1. The level of daily physical activity, and 2. The level of program activity (exercises). The answers were coded categorically: 0 – inactivity; 1 – subthreshold activity; 2 – threshold activity; 3 –suprathreshold activity. The scores of questions 1 and 2 are summed and give the scale score as a whole (0 and 1 – unsatisfactory level of physical activity; score ≥ 2 – satisfactory level of physical activity) [18].

Symptoms of depression

The symptoms of depression were assessed using Patient Health Questionnaire-9 (PHQ-9), which was validated as a screening instrument for use in Primary Health Care in Serbia. The questionnaire consists of 9 questions about symptoms and signs of depression. According to achieved scores, the subjects were classified into four groups: absence of symptoms of depression (score 0–4); mild symptoms of depression (score 5–9); moderate symptoms of depression (score 10–14); moderate to severe symptoms of depression (score 15–27) [19].

DM-related distress

The DM-related distress was assessed by Diabetes Distress Scale. The questionnaire differentiates three groups of subjects: with little or no distress (score > 2); with moderate distress (score = 2–2.9); with high, clinically significant distress (score > 3) [20]. The questionnaire showed good internal consistency (Cronbach α = 0.93).

Statistical analysis

Statistical data processing was performed using the statistical program IBM SPSS 21.0. Data processing included methods of descriptive and inferential statistics. Numerical features are presented through mean values (arithmetic mean), measure variability (range of values and standard deviation), and attributive features using frequencies and percentages. A binary logistic regression model was used to examine the correlation and prediction of the dependent variable defined as dichotomous. The odds ratio (OR) was used in the interpretation of the model together with a 95% confidence interval (CI). As a measure of the model's adaptation to real data, the Hosmer-Lemeshow test was used to test the differences between the observed and expected values. The sensitivity and specificity of the model and the total percentage of correctly classified respondents based on the model were used in the interpretation of the results. All tests were two-sided with a significance level of $p < 0.05$.

Results

Demographic and clinical characteristics

The mean age of subjects in the research sample was 63.8 ± 9.3 years. The largest number of respondents belonged to the age group 45–65 years (n = 155; 47.8%). The demographic characteristics of respondents stratified by gender are shown in Table 1.

Most of the subjects in the sample (n = 207; 63.9%) had DM 1–10 years, and the average duration of the disease in the sample as a whole was 11.0 ± 8.3 years. The average BMI of subjects in the sample was 31.3 ± 5.7 kg/m^2 indicating obesity. Only 38 (11.1%) respondents in our study sample were without complications or comorbidities. The clinical characteristics of respondents stratified by gender are shown in Table 2.

Table 1

Demographic characteristics of patients with type 2 diabetes mellitus stratified by gender

Characteristic	Male		Female		Total	
	n	%	n	%	n	%
Number of respondents	141	43.5	183	56.5	324	100.0
Age categories (years)						
30–45	11	7.8	6	3.3	17	5.2
46–65	69	48.9	86	47.0	155	47.8
> 65	61	43.3	91	49.7	152	46.9
Average age (years), mean ± SD	62.6 ± 9		64.7 ± 9.2		63.8 ± 9.3	
Marital status						
live alone	111	78.7	115	62.8	226	69.8
community life	30	21.3	68	37.2	98	30.2
Education						
elementary/lower	29	20.6	93	50.8	122	37.7
high school	86	61.0	73	39.9	159	49.1
college/university	26	18.4	17	9.3	43	13.3
Financial status						
poor	45	31.9	100	54.6	145	44.8
satisfactory	74	52.5	68	37.2	142	43.8
good	22	15.6	15	8.2	37	11.4

SD – standard deviation.

Table 2

Clinical characteristics of patients with type 2 diabetes mellitus (DM) stratified by gender

	Male		Female		Total	
	n	%	n	%	n	%
Number of respondents	141	43.5	183	56.5	324	100.0
Duration of DM (years)						
1–10	90	63.8	117	63.9	207	63.9
11–20	34	24.1	42	23.0	76	23.5
> 20	17	12.1	24	13.1	41	12.7
Average duration (years), mean ± SD	10.7 ± 8.1		11.1 ± 8.5		11.0 ± 8.3	
The presence of other diseases (n = 286)						
only comorbidities	52	44.1	75	44.6	127	44.4
comorbidities and complication	57	48.3	85	50.6	142	49.6
only complication	9	7.6	8	4.7	17	5.9
BMI (kg/m^2), mean ± SD	29.9 ± 4.7		32.3 ± 6.2		31.3 ± 5.7	
Therapeutic modality						
tablets	78	55.3	100	54.5	178	54.9
insulin	29	20.6	43	23.4	72	22.2
tablets + insulin	34	24.1	40	22.1	74	22.8
Dose regimens						
1 dose	14	9.9	21	11.5	35	10.8
2 doses	65	46.1	86	47.0	151	46.7
≥ 3 doses	62	43.9	76	41.5	138	42.5
Is insulin an additional burden (n = 146)?						
yes	33	52.4	69	83.1	102	69.4
no	30	47.6	15	16.9	45	30.6
How does insulin therapy burden you (n = 102)?						
fear of hypoglycemia	6	18.2	24	34.8	30	29.4
limiting the activity of everyday life	19	57.6	25	36.2	44	43.1
frequent checking of blood glucose levels	8	24.2	20	29.0	28	27.5

SD – standard deviation; BMI – body mass index; DM – diabetes mellitus.

Medication adherence

Slightly more than half of the respondents (n = 169; 55.2) had positive attitudes toward medication adherence, while 155 (47.8%) had negative attitudes. The results of the univariate analysis showed that female gender ($p = 0.001$), respondents < 65 years of age ($p = 0.009$), elementary or lower level of education ($p < 0.001$), poor financial status ($p = 0.009$), obesity ($p < 0.001$), insulin therapy with load ($p < 0.001$), and ≥ 3 therapeutic doses daily ($p < 0.001$) were statistically significantly related to negative attitudes toward therapeutic adherence.

Adherence to dietary recommendations

By dichotomizing the total value of the PDQ score (cut-off = 37), almost two-thirds of the respondents (n = 211; 65.1%) rated themselves as non-adherent toward dietary recommendations. Non-adherence was more common in persons < 65 years compared to the group > 65 years (71.5% vs. 57.9%, respectively) and in obese persons compared to those with normal body mass (74.7% vs. 36.4%, respectively). The duration of diabetes ($p = 0.772$) was not statistically associated with adherence to dietary recommendations.

Physical activity

The results obtained using the subscale of Physical activity in PDQ indicate that more than half of respondents (n = 211; 65.1%) had unsatisfactory levels of physical activity. The results of the univariate analysis showed that the female gender ($p = 0.027$), elementary or lower level of education ($p = 0.001$), obesity ($p < 0.001$), and the presence of complications ($p < 0.001$) were statistically significantly related to the unsatisfactory level of physical activity.

Symptoms of depression

In our study population, every second respondent had mild to moderate depression (n = 154; 50.6%), every tenth (n = 33; 10.2) had moderate to severe depression, and two-fifths had no symptoms of depression. The results of the univariate analysis showed that the female gender ($p < 0.001$), elementary or lower level of education ($p = 0.003$), insulin therapy with load ($p = 0.001$), and the presence of complications ($p = 0.002$) were statistically significantly related to symptoms of depression.

DM-related distress

By dichotomizing the value of total scores and scored associated subscales into categories of clinically significant distress (score ≥ 3) and no distress or moderate distress without clinical significance (score < 3), we obtained the results that clinically significant distress was present in 114 (35.2%) respondents. The results of the univariate analysis showed that the female gender ($p = 0.006$), higher levels of education ($p = 0.029$), insulin therapy with load ($p < 0.001$), three and more therapeutic doses daily ($p = 0.031$), and the presence of complication ($p = 0.005$) were statistically significantly related to clinically significant distress.

Relationship and predictive influence of examined variables on the metabolic control of subjects with T2DM

Slightly less than a quarter of respondents in the research sample met all three goals of good metabolic control (n = 70; 21.6%). Data on the values of metabolic control parameters of the subjects in our study sample are shown in Table 3.

A binary logistic model was analyzed in order to determine the correlation and prediction of good metabolic control defined by achieving the target values of all three metabolic parameters. The first step was the application of univariate analysis with a dichotomized dependent variable metabolic control (good – met all three goals and bad – not met all three goals). Some socio-demographic and clinical characteristics of respondents, behavioral determinants of DM self-management, and psychological characteristics of the respondents (symptoms of depression and DM-related distress) were applied as dependent variables in the model. Detailed data are shown in Table 4.

The results of the univariate analysis showed that the metabolic control of subjects could be associated with several variables between which there may be an independent relationship. Multivariate logistic regression analysis showed which of these variables can be independent predictors as opposed to others that affect metabolic control as cofactors. The results are shown in Table 5.

Table 3

Metabolic control parameters of patients with type 2 diabetes mellitus (n = 324, 100%)

Parameters	Value
Blood pressure, n (%)	
normal blood pressure	195 (60.2)
arterial hypertension	129 (39.8)
Hb_{A1C} (%), mean values ± SD	7.5 ± 1.5
Regulation of blood glucose, n (%)	
good	126 (38.9)
bad	198 (61.1)
HDL(mmol/L), mean ± SD	1.3 ± 0.3
LDL (mmol/L), mean ± SD	3.2 ± 1.1
Triglycerides (mmol/L), mean ± SD	2.2 ± 1.5
Lipid status, n (%)	
normolipidemia	113 (34.9)
dyslipidemia	211 (65.1)

Hb_{A1C} – glycosylated hemoglobin; SD – standard deviation; HDL – high-density lipoproteins; LDL – low-density lipoproteins.

Table 4

Metabolic control in relation to demographic and clinical characteristics, behavioral determinants of diabetes mellitus (DM) management, and psychological characteristics of respondents (univariate analysis)

Parameter	Metabolic control				*p*
	good		bad		
	n	%	n	%	
Number of respondents	70	21.6	254	75.4	
Gender	40	28.4	101	71.6	$\chi^2 = 6.743$
male	30	16.4	153	83.6	$p = 0.009$
female					
Age categories (years)					
30–65	31	18.0	141	82.0	$\chi^2 = 2.777$
> 65	39	25.7	113	74.3	$p = 0.008$
Body mass level					
normal	22	50.0	22	50.0	$\chi^2 = 32.986$
overweight	28	26.4	78	73.6	$p < 0.001$
obesity	20	11.5	154	88.5	
Duration of DM (years)					
1–20	46	22.2	161	77.8	$\chi^2 = 0.129$
> 20	24	20.5	93	79.5	$p = 0.720$
Insulin therapy					
no	54	30.3	124	69.7	$\chi^2 = 19.424$
yes, no load	8	17.4	38	82.6	$p < 0.001$
yes, with load	8	8.0	92	92.0	
Number of therapeutic doses					
1	55	29.3	133	70.7	$\chi^2 = 15.477$
2 or more	15	11.0	121	89.0	$p < 0.001$
Complications					
no	40	24.2	125	75.8	$\chi^2 = 1.381$
yes	30	18.9	129	81.1	$p = 0.240$
Dietary adherence					
yes	48	42.5	65	57.5	$\chi^2 = 44.643$
no	22	10.4	189	89.6	$p < 0.001$
Depression					
no depression/minimal	36	28.3	91	71.7	$\chi^2 = 5.605$
yes	34	17.3	163	82.7	$p = 0.018$
DM related distress					
no/moderate without clinical significance	58	27.6	152	72.4	$\chi^2 = 12.746$
clinically significant	12	10.5	102	89.5	$p < 0.001$
Level of physical activity					
satisfactory	51	35.7	92	64.3	$\chi^2 = 29.874$
unsatisfactory	19	10.5	162	89.5	$p < 0.001$
Medication adherence					
adherent	70	41.4	99	58.6	$\chi^2 = 81.849$
non-adherent	0	0.0	155	100.0	$p < 0.001$

Table 5

Logistic regression model with poor metabolic control as dependent variable

Independent variables	p^*	OR (95% CI)
Body mass level	< 0.001	
normal		1.00
overweight	0.018	2.80 (1.20, 6.55)
obesity	< 0.001	5.61 (2.36, 13.35)
Insulin therapy	0.001	
no		1.00
yes/no load	0.008	3.68 (1.41, 9.60)
yes/with load	0.004	3.73 (1.54, 9.04)
Dietary adherence		
good		1,00
bad	< 0.001	3.73 (1.95, 7.15)
Diabetes mellitus related distress		
no distress/moderate with no clinical significance		1.00
clinically significant distress	0.040	2.26 (1.04, 4.93)
Level of physical activity		
satisfactory		1,00
unsatisfactory	0.004	2.73 (1.39, 5.35)

The value of Hosmer-Lemeshow (C = 8.318, *p* = 0.403) indicates the agreement of the model with the data. The sensitivity of the model shown is 93.3%, the specificity is 37.1%, and the overall accurate prediction is 81.2%.

***– the Wald test; OR – odds ratio; CI – confidence interval.**

Discussion

In the conducted research, all three goals of metabolic control were met by 21.6% of respondents, which is significantly better than the results of studies conducted in Japan (11.2%) [21] and Poland (8%) [22]. Females in this study had poorer metabolic control than males, which is consistent with the results of studies published by others [23]. The higher prevalence of psychological distress (symptoms of depression and DM-related distress), negative attitudes toward medication adherence, and a lower level of physical activity caused by traditional social roles of this gender group may be an explanation for this finding.

An interesting finding is that older people (> 65 years) have better metabolic control than people < 65 years. The same age distribution of metabolic control is indicated by the results of other studies [4, 21]. The finding can be interpreted as a higher life load of younger people, less tendency to change ingrained life habits, and greater sensitivity to the stigma of the disease.

Results of the conducted research indicate a negative relationship between attitudes toward medication adherence and metabolic control of the patients with T2DM. Subjects with lower Attitude Scale scores (poorer medication adherence) also show poorer metabolic control of T2DM. The published research results in the available literature are consistent with ours [24]. T2DM medication therapy is often complex since it required a higher number of drugs and higher daily doses. In our study, 37.7% of respondents had a lower level of education, and 44.8% assessed their financial situation as unsatisfactory. For this reason, the lack of knowledge about the disease, the importance of therapy, and the poor availability of drug therapy may explain the negative association between metabolic control and attitudes toward medication adherence.

In our study, complex therapeutic regimens (42.5% of respondents) showed a statistically significant association with negative attitudes toward drug adherence. That, as well as the fact that complex therapeutic regimens can create difficulties in integrating determinants in the management of DM into daily life routines [25], can be explained by this finding.

Data from the literature on the relationship between depressive symptoms and metabolic control parameters are inconsistent. Authors who are consistent with our results find a positive correlation between depressive symptoms and metabolic control parameters [26], while others do not [27]. The differences observed may be methodological in nature and depend on questionnaires for assessing the symptoms of depression used in the research.

The study finding of the negative predictive effect of overweight [odd ratio (OR) = 2.80; 95% confidence interval (CI) = 1.20–6.55)] and obesity (OR = 5.61; 95% CI = 2.36–13.35) on metabolic control is consistent with the findings of other studies [28]. In contrast, other studies have not found an association of obesity with any metabolic control parameters [4]. Obesity increases insulin resistance and glucose intolerance, as well as sympathetic activity [29], worsening all metabolic control parameters of individuals with T2DM, which explains this finding.

The study finding of the negative predictive effect of insulin therapy on metabolic control (OR = 3.68 – no load; OR = 3.73 – with load) is consistent with the findings of other studies [23]. This remarkable finding can be explained by understanding that the introduction of insulin into therapy for people with T2DM means that the disease is in a phase that is difficult to control. That leads to clinically significant distress with a negative impact on metabolic control. Furthermore, fear of hypoglycemia is present in 29.4% of our subjects on insulin therapy, leading them to take larger amounts of food and reduce physical activity in order to avoid hypoglycemia. One explanation for this finding worth considering in future research is that DM-related distress is a major barrier to initiating insulin therapy and that clinical inertia in the introduction of insulin into T2DM therapy is one of the reasons for poorer metabolic control in this group of patients [30].

The results of the multivariate logistic regression analysis conducted in this study indicate the predictive effect of an unsatisfactory level of physical activity (OR = 2.73; 95% CI = 1.39–5.35) on poor metabolic control of subjects with T2DM, which is consistent with the findings of other studies [31]. The results of the Look AHEAD study indicate this indirectly, showing that weight loss reduces the need for drugs that regulate glycemic status, BP, and lipid status [14].

In this study, clinically significant DM-related distress stands out as a significant predictor of poor metabolic control (OR = 2.26; 95% CI = 1.29–5.35). DM-related distress can affect metabolic control directly through pathophysiological processes (hypothalamic-pituitary axis activation, increased sympathetic activity, and insulin resistance) and indirectly through DM self-management, which may explain these findings [24].

Limitations and adventages of the study

The advantage of studies of this type is that they enable proper control of measurements and assessment of the prevalence of research determinants. However, the key disadvantage of these studies is the impossibility of gaining insight into the time sequence of the examined phenomena, i.e., it is not possible to determine the direction of causality for which longitudinal studies are necessary.

As self-completing questionnaires are used as research instruments, recall bias and giving socially desirable answers could not be completely avoided. The construction of individual questionnaires enabled bias of the central tendency, i.e., giving answers that were in the middle of the scale of offered answers.

Despite the existence of these limitations, the representativeness of the sample, random sampling methods, and the use of internationally recognized standardized research instruments give significant strength to the conducted study.

Conclusion

This study provides insight and understanding of a wide range of issues in the context of self-management of DM, which is crucial for achieving a much more effective approach to patients with T2DM. The available therapeutic modalities are less likely to be effective in individuals who have difficulty adhering to behavioral determinants of DM self-management because these problems are often beyond the reach and influence of the physicians dealing with the medical treatment of persons with T2DM.

The application of questionnaires used in this study in the care of patients with T2DM, at all levels of health care, in the initial stages or critical moments of the disease, provides insight into their knowledge and attitudes about behavioral determinants of DM self-management and timely detection of psychological conditions that affect them. That is the basis of the necessary multidisciplinary approach to patients with T2DM, which, by including other specialties (psychologists, nutritionists, physiotherapists), provides support to patients through education, motivation, behavior modification, and psychological support. The results of conducted research indicate that it is reasonable to expect that such a comprehensive approach contributes to better metabolic control of patients with T2DM.

The study results indicate that longitudinal research is necessary for a better understanding of the impact of the research determinants and for evaluating the effectiveness of a multidisciplinary approach in achieving better metabolic control of patients with T2DM. That is a basic recommendation for future research.

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Antithrombin activity as a significant predictor of early mortality in pulmonary embolism patients

Antitrombinska aktivnost kao značajan prediktor ranog mortaliteta kod bolesnika sa embolijom pluća

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Abstract

Background/Aim. The role of antithrombin (AT) activity in predicting early mortality in patients with pulmonary embolism (PE), measured at an early stage of the disease, has not yet been investigated. Therefore, the aim of the study was to examine the predictive value of AT activity for all-cause 30-day mortality, measured in consecutive PE patients on admission to the hospital. **Methods**. This single-center clinical retrospective cross-sectional study followed consecutive patients with acute PE from 2014 to 2021. On admission to the hospital, venous blood was taken from patients for laboratory analyses including determination of AT activity. The basic parameters of the patients were recorded on admission, and through the univariate analysis, their connection with 30-day mortality was tested. The predictive significance of AT values for 30-day mortality was tested through quartile values by comparing the first quartile with all others together. Cox regression model analysis was used in the multivariate analysis where one parameter, marked as significant in the univariate analysis, was added to the basic model (AT, age, and risk affiliation in two groups). **Results**. A total of 378 PE patients were included in the study. The total all-cause 30-day mortality was 7.9% (30 patients). Patients with AT activity in the first quartile had significantly higher early mortality compared with those having AT activity in the other quartiles combined (log-rank $p = 0.001$). AT retained a significant predictive value for early mortality in the multivariate analysis despite the comorbidity present, which also significantly affected mortality. **Conclusion**. Low AT activity measured on admission in PE patients is a significant and independent predictor of 30-day mortality.

Key words:
antithrombins; mortality; prognosis; pulmonary embolism.

Apstrakt

Uvod/Cilj. Uloga aktivnosti antitrombina (AT) u predviđanju rane smrtnosti kod bolesnika sa plućnom embolijom (PE), merena u ranoj fazi bolesti, još uvek nije istražena. Cilj rada bio je da se ispita prediktivna vrednost aktivnosti AT za 30-dnevni mortalitet, merena na prijemu, kod bolesnika sa PE. **Metode**. Retrospektivnom studijom preseka, praćeni su svi bolesnici sa akutnom PE, lečeni u našoj Klinici u periodu od 2014. do 2021. godine. Bolesnicima je na prijemu uzimana venska krv za laboratorijske analize uključujući i merenje aktivnosti AT. Osnovni podaci o bolesnicima beleženi su na prijemu, a primenom univarijantne analize testirana je njihova povezanost sa 30-dnevnim mortalitetom. Prediktivni značaj vrednosti AT za 30-dnevni mortalitet testiran je kroz kvartilne vrednosti tako što je prvi kvartil poređen sa svim ostalima zajedno. *Cox*-regresiona analiza korišćena je u multivarijantnoj analizi, gde je osnovnom modelu (AT, godine i pripadnost riziku u dve grupe) dodavan po jedan parametar, označen kao značajan u univarijantnoj analizi. **Rezultati**. U studiju je bilo uključeno ukupno 378 bolesnika sa PE. Ukupan 30-dnevni mortalitet iznosio je 7.9% (30 bolesnika). Bolesnici sa aktivnošću AT u prvom kvartilu imali su značajno veći rani mortalitet u poređenju sa onima koji su tu aktivnost imali u ostalim kvartilima zajedno (*log rank* $p = 0{,}001$). Varijabla AT je zadržala značajnu prediktivnu vrednost za rani mortalitet i u multivarijantnoj analizi bez obzira na prisutni komorbiditet, koji je takođe značajno uticao na mortalitet. **Zaključak**. Niska aktivnost AT izmerena na prijemu kod bolesnika sa PE je značajan i nezavisan prediktor 30-dnevnog mortaliteta.

Ključne reči:
antitrombini; mortalitet; prognoza; pluća, embolija.

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Introduction

Pulmonary embolism (PE) is the third cause of cardiovascular mortality, just behind acute myocardial infarction and stroke. The mortality risk from PE is highest in the first 30 days and gradually decreases over time, reaching the mortality rate of a normal population. The overall mortality rate from PE is around 9.7% but could vary significantly depending on associated comorbidity. For instance, the mortality rate of PE patients with malignancy is 19.1%, but only 3.6% in those without malignancy [1]. The current guideline suggests the assessment of early mortality risk prior to choosing the optimal treatment strategy [2]. According to the newest guideline, patients who are hypotensive or in shock should be treated with reperfusion therapy, such as fibrinolysis, or catheter-guided and surgical thrombectomy. PE patients who are not hypotensive, even with imaging or laboratory signs of increased right ventricular (RV) load, should be anticoagulated and closely monitored, and reperfusion therapy should be used only if clinical deterioration occurs. Previous studies reported higher survival of such PE patients (with intermediate-high risk PE) who were treated initially with fibrinolysis but with higher cost because of increased major bleeding [3–5]. Therefore, additional parameters for assessing early mortality in PE are warranted in order to identify PE patients who are at risk of sudden deterioration and who would benefit from immediate reperfusion therapy.

Antithrombin (AT) is a main natural anticoagulant, and its acquired deficiency has already been reported in published data of patients with massive PE [6]. However, the predictive value for early mortality in PE of such acquired AT deficiency has not been investigated so far. The aim of the study was to test the predictive value of AT for 30-day mortality in consecutive PE patients through univariate and multivariate analyses, including the most present comorbidities.

Methods

A single-center retrospective cross-section study included all consecutive PE patients admitted to the Clinic for Emergency Internal Medicine at the Military Medical Academy in Belgrade, the Republic of Serbia, from November 2014 to May 2021. The study was approved by the institutional Ethics Committee (No 160/2019, from December 26, 2019). Diagnosis of PE was confirmed with multi-detector CT pulmonary angiography in all patients. All patients were treated with the highest regard as per current guidelines [2]. Using 30-day mortality risk as a baseline, PE patients were classified as high-risk (presenting with shock or hypotension where systolic pressure was below 90 mmHg, or normotensive patients with signs of RV dysfunction on transthoracic echocardiography or increased troponin in laboratory analysis) or low-risk (normotensive PE patients with no signs of RV dysfunction). Patients' baseline characteristics were obtained on admission. Antecubital venipuncture was used to collect blood samples for full blood count and laboratory analysis of troponin I (TnI), C-reactive protein (CRP) levels, and activity of AT. Blood samples were collected after the initial *iv* bolus of unfractionated heparin and prior to eventual thrombolytic treatment. Except for the initial anticoagulation with heparin, all patients were not on anticoagulant treatment before admission. The initial dose of heparin was 80 U/kg but did not exceed 5,000 U, as per protocol for the treatment of PE. The glomerular filtration rate (GFR) was calculated using the Cockcroft-Gault equation. Laboratory analyses for AT activity were performed using an automatic coagulometer, type BCS-XP Siemens Healthcare Diagnostics Products GmbH (Marburg, Germany). All baseline characteristics were compared in univariate analysis between the group of PE patients who died in the first 30 days and those who survived. When a significant association with early mortality was identified among tested characteristics, a Cox regression model analysis was used in a multivariate analysis of AT predictive value. AT, age, and risk stratification were used in a baseline model of a Cox regression analysis, and other co-factors were added separately, building different models that represented different associated comorbidity. When AT activity values were assessed as a predictor of early mortality, a quartile with values associated with increased frequency of the observed outcome was compared with other quartiles together.

Statistical analysis

Categorical variables were expressed as frequencies and percentages, and continuous variables as means with standard deviations. For values without normal distribution, the nonparametric Mann-Whitney *U* or Kruskal-Wallis H tests were used, with the results expressed as the median of the interquartile range. A Hazard Ratio (HR) was calculated for the observed outcome using multivariable Cox regression model analysis. Optimal cut-off values and their sensitivity and specificity, along with positive and negative predictive values, for the prediction of 30-day mortality were calculated in MedCalc for Windows version 12.7.0.0 (MedCalc Software, Acacialaan, Belgium). Comparisons of nonparametric variables and frequencies of categorical data between survivors and deceased patients were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Differences with a *p*-value of ≤ 0.05 were considered significant.

Results

During the follow-up period, 378 PE patients overall entered the analysis. Among them, 191 (50.5%) were men, and 187 (49.5%) were women. All-cause 30-day mortality was 30 patients (7.9%). Increased mortality was noticed in patients with unprovoked PE, chronic heart failure, coronary artery disease, and atrial fibrillation. Additionally, PE patients with diabetes mellitus (DM), chronic kidney disease, and malignancy had also increased early mortality. Other patients' characteristics on admission to the hospital and their relation to the 30-day mortality rate are shown in Table 1.

The median value of AT activity, measured on admission, in PE patients who died in the first 30 days was significantly lower than the value in patients who survived [0.73

(0.60–0.96) vs. 0.89 (0.78–0.99), respectively; $p = 0.002$]. Additionally, PE patients with AT activity values within the first quartile had significantly lower survival as compared with the patients who had AT activity values within other quartiles (Figure 1).

A cut-off value of AT activity for increased early mortality rate was ≤ 0.8 U/L (AUC = 0.670, $p = 0.008$) with 66.7% sensitivity and 70.7% specificity. The positive predictive value was 16.4%, and the negative predictive value was 96.1% (Figure 2).

Table 1

Baseline characteristics of the pulmonary embolism (PE) patients and 30-day mortality

Characteristic	30-day mortality		*p*
	yes n = 30	no n = 348	
Age (years), mean ± SD	69 ± 15	60 ± 16	**0.003**
Sex, n (%)			0.257
men	12 (40.0)	179 (51.4)	
women	18 (60.0)	169 (48.6)	
Factors that provoked PE, n (%)			**< 0.001**
unprovoked PE	9 (30.0)	189 (54.3)	
major transient	4 (13.3)	61 (17.5)	
minor persistent	9 (30.0)	36 (10.3)	
minor transient	1 (3.3)	35 (10.1)	
major persistent	7 (23.3)	27 (7.8)	
COPD, n (%)	1 (3.3)	24 (6.9)	0.708
CHF, n (%)			**< 0.001**
HFrEF	3 (10.0)	5 (1.4)	
HFmrEF	4 (13.3)	14 (4.0)	
HFpEF	4 (13.3)	21 (6.0)	
Smokers, n (%)	3 (10.0)	65 (18.7)	0.272
Obesity (BMI > 30 kg/m^2), n (%)	5 (16.6)	102 (29.3)	0.278
Localization of DVT, n (%)			0.964
distal	13 (44.4)	181 (52.0)	
proximal	17 (56.6)	162 (46.6)	
Arterial hypertension, n (%)	21 (70.0)	187 (53.7)	0,125
Coronary artery disease, n (%)	7 (23.3)	28 (8.0)	**0.013**
Diabetes mellitus, n (%)	9 (30.0)	51 (14.7)	**0.037**
Atrial fibrillation, n (%)			**< 0.001**
paroxysmal	10 (33.3)	26 (7.5)	
permanent	2 (6.7)	15 (4.3)	
Creatinine clearance < 30 mL/min, n (%)	5 (16.7)	16 (4.6)	**0.019**
Creatinine clearance < 60 mL/min, n (%)	15 (50.0)	79 (22,7)	**0.003**
Malignancy, n (%)	9 (30.0)	40(11.5)	**0.007**
CRP (mg/L), median (IQR)	95.9 (59.5–161.1)	44.7 (19.0–108.0)	**< 0.001**
Total leukocyte count (×10^9), median (IQR)	12.7 (10.3–15.6)	9.7 (7.6–12.5)	**< 0.001**
Fibrinolysis, n (%)	13 (43.3)	168 (48.3)	0.704

COPD – chronic obstructive pulmonary disease; CHF – chronic heart failure; HFrEF – heart failure with reduced ejection fraction (EF); HFmrEF – heart failure with mid-range EF; HFpEF – heart failure with preserved EF; BMI – body mass index; CRP – C-reactive protein; IQR – interquartile range; SD – standard deviation.
Statistically significant values are bolded.

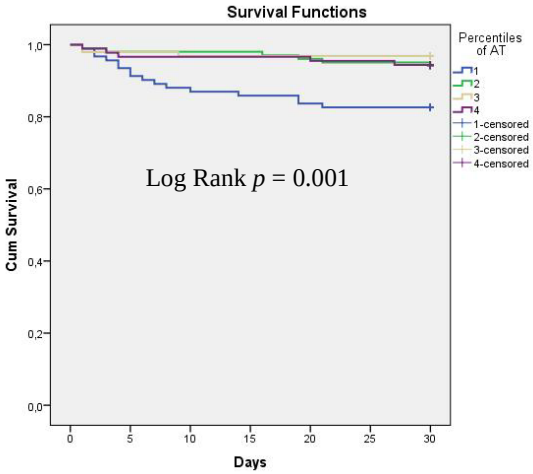

Fig. 1 – Kaplan-Meier survival curve regarding quartiles of antithrombin (AT).

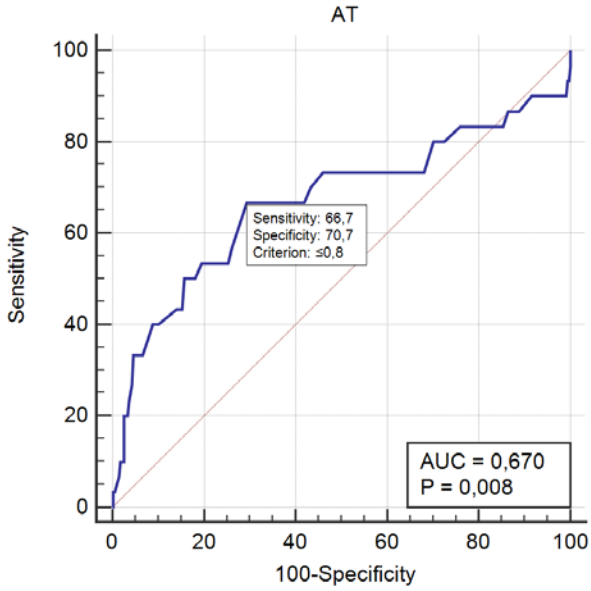

Fig. 2 – Receiver operating characteristic (ROC) curve depicting the cut-off value of antithrombin (AT) activity for increased early mortality. AUC – area under curve.

Table 2

Predictive value of antithrombin (AT) and adjusted hazard ratio (HR) for 30-day mortality

Adjusted to	HR (95% CI) of AT as a 30-day mortality predictor	*p*
Malignancy, age, and risk in two groups	2.75 (1.25–6.06)	0.012
Diabetes mellitus, age, and risk in two groups	2.75 (1.33–5.68)	0.006
HFrEF, age, and risk in two groups	2.58 (1.16–5.70)	0.020
Coronary artery disease, age, and risk in two groups	2.80 (1.36–5.79)	0.005
Atrial fibrillation, age, and risk in two groups	2.50 (1.21–5.18)	0.013
Chronic kidney disease (CrCl < 60 mL/min), age, and risk in two groups	2.43 (1.15–5.14)	0.006
Inflammation (CRP and TLC), age, and risk in two groups	2.45 (1.15–5.18)	0.020
Fibrinolysis and age	3.36 (1.61–6.99)	0.001

HFrEF – heart failure with reduced ejection fraction; CrCl – creatinine clearance; CRP – C-reactive protein; TLC – total leukocyte count; CI – confidence interval.

Cox proportional hazards model showed that AT was a significant predictor of early mortality when adjusted to age and risk in two groups as co-factors. When different comorbidities, shown to be significantly associated with early mortality, were included in this basic model, AT remained a significant and independent predictor of this outcome. When the patients who received fibrinolysis were included in the Cox proportional hazard model (without the risk in two groups as a co-factor due to possible bias), AT remained a significant predictor of 30-day mortality (Table 2).

Discussion

The results of this study have shown that AT activity, measured in PE patients on admission to the hospital, can serve as a reliable early mortality predictor in addition to other conventional predictors of 30-day mortality in PE, already recommended in the current guidelines.

The patients diagnosed with PE may have additional comorbidity that can impact survival. Some of those comorbidities are included in the Pulmonary Embolism Severity Index (PESI) score, which is involved in the assessment of early mortality risk together with imaging and laboratory signs of an increased RV load [2]. However, the results of this study showed that some other comorbidity, not included in the PESI score, may exist in PE patients but still be associated with early mortality, such as DM, chronic kidney disease, atrial fibrillation, coronary artery disease, or markers of inflammation. When adjusted to age and affiliation to one of the two risk groups for early mortality (determined by the presence of clinical and laboratory signs of an increased RV load), AT was a significant predictor of early mortality. When added independently to this Cox proportional hazard model, whether it was malignancy, diabetes, chronic kidney disease, atrial fibrillation, coronary artery disease, or markers of inflammation (CRP and total leukocyte count), AT remained an independent and significant predictor. Furthermore, some of the patients received fibrinolysis in their treatment, which probably had an impact on their survival. Nevertheless, when adjusted to age and fibrinolysis, AT was

still a significant predictor of early mortality. Hence, AT activity measured early in the course of PE can indicate the increased risk of early mortality, independently of other predictors recommended in the guidelines, and thus may influence the decision for optimal therapy. That is especially important in patients with intermediate-high risk PE where routine administration of fibrinolysis is not advised. According to the current guideline, it is advised that patients' subgroup with intermediate-high risk PE be carefully monitored and fibrinolysis be administered only in case of clinical deterioration [2]. According to the results of this study, PE patients with AT activity in the first quartile had a significantly increased 30-day mortality rate. Given that the values of AT in the first quartile are relatively rare, we determined the cut-off value of AT activity for increased mortality rate, which was ≤ 0.8 U/L and could be useful for the clinician when assessing the mortality risk. Hence, low AT activity may indicate increased early mortality risk in intermediate-high risk PE and, therefore, facilitate early administration of fibrinolysis in such patients.

Low AT activity in massive PE could be due to the consumption of AT during the excessive thrombus formation leading to an acquired AT deficiency. Such theory was first implicated by Leitner et al. [6], who have demonstrated that patients with massive PE requiring cardiopulmonary resuscitation have reduced AT levels as compared with PE patients not requiring cardiopulmonary resuscitation. Furthermore, PE patients resuscitated for reasons other than PE (i.e., resuscitation as a result of primary cardiac causes) have a markedly less intense activation of coagulation than patients with PE. Hence, a pulmonary clot itself may likely contribute to coagulopathy. AT has the role of scavenger because it can inhibit free thrombin and free factor Xa more efficiently than it inhibits thrombin and factor Xa located on activated surfaces and in fibrin clots [7]. That may be because this function of AT blocks coagulation and thrombin non-coagulant functions in remote and uninjured areas. The clearance of AT from the circulation by the liver is more rapid when it is in the form of thrombin–AT (TAT) complex [7]. Therefore, in clinically severe thrombosis and thromboembolism, more TAT complexes are formed. The more TAT complexes are formed, the more rapidly AT is cleared from circulation by the liver.

In this study, PE patients with malignancy and low AT activity had also increased early mortality. Earlier studies demonstrated the bidirectional interaction of AT and malignant cells. It was noticed that patients with malignancy have lower AT activity. Furthermore, AT was found to be an inhibitor of metastasis of particular tumors, and some of the tumors can even secrete inhibitors of AT [8–11]. Therefore, decreased AT in malignancy is not only due to consumption but as a consequence of direct AT-tumor interaction.

Cardiovascular comorbidities are the most frequent comorbidities in PE patients [12]. In our study, the most frequent were heart failure, in particular heart failure with reduced ejection fraction (HFrEF), atrial fibrillation, and coronary artery disease. Low values of AT were significant predictors in PE patients with these comorbidities. Interestingly, the low value of AT was also recognized as a significant predictor of cardiovascular mortality.

DM is another frequent comorbidity in PE patients. Scherz et al. [13] stated in their research on over 13,000 PE patients that DM was an independent predictor of early mortality. In fact, they found that stress hyperglycemia was a more precise parameter for mortality assessment and that this glycemic increase measured on admission was more indicative of early mortality in non-diabetics than in diabetics. The existence of DM or chronic hyperglycemia, as well as acute (stress) hyperglycemia, is a known prothrombotic factor that may increase the incidence of venous thromboembolism (VTE) [14, 15]. Numerous published studies have shown that AT activity was decreased both in DM type 1 and 2 [16–18]. The state of hyperglycemia has a strong impact on AT, decreasing its activity. In addition, these studies have shown that normalization of glycemic levels can reverse AT activity. One of the main inhibitors of AT activity identified in hyperglycemia is methylglyoxal. Therefore, in our PE patients with DM, stress hyperglycemia may be one of the mechanisms for acquired AT deficiency and increased mortality.

Various published studies demonstrated an increased risk of PE in patients with chronic kidney disease [19, 20]. In our study, 50% of patients with PE who died in the first 30 days had creatinine clearance below 60 mL/min. Kumar et al. [20] reported that the mortality rate of PE patients with chronic kidney disease was 57% higher than in PE patients without chronic kidney disease. The main mechanism of hypercoagulability in chronic kidney disease is through the increase of tissue factor, coagulation factor VII, XII, and fibrinogen, and decrease of tissue plasminogen inhibitor [21–23]. With a progression of kidney failure toward the terminal phase, there is a larger increase of fibrinogen, thrombin–AT complexes, and acquired protein C (PC) deficiency [24, 25]. In chronic kidney disease with nephrotic syndrome, increased daily loss of AT is the main reason for hypercoagulability [26].

In the last decade, there have been a lot of published data regarding the increased coagulability in inflammation. The baseline mechanism lies in the activation of endothelial cells, platelets, and leukocytes, which leads to the increased formation of tissue factor-rich microvesicles. Furthermore, when inflammation is present, fibrinogen increases, thrombomodulin decreases, and there is an increase in plasminogen activator inhibitor-1 (PAI-1) (leading to decreased fibrinolysis). The consequence of such a process is a hypercoagulable state and a decrement of thrombus resolution [27–29]. The role of neutrophils in thrombosis is also important. When activated, neutrophils can release their decondensed chromatin to extracellular space, forming traps (neutrophil extracellular traps – NETs) for microorganisms, but also promoting thrombosis through activation of platelets and coagulation [30, 31]. In our study, CRP and leukocyte count were used as markers of inflammation and so used in a multivariant analysis of mortality prediction. AT was a significant predictor of early mortality in such a multivariant model. Low activity of AT in inflammation is due to increased removal of AT from the circulation by activated neutrophils. They secret neutrophil elastase and matrix metalloproteinases that cleave

the active loop on AT molecule, which inactivates AT [7]. Therefore, in PE and inflammation, more mechanisms are involved in the occurrence of an acquired AT deficiency.

The clinical implication of the results from this study could be in better understanding of the role of AT in mortality prediction in acute PE where different comorbidities are present. Low AT activity represents a higher mortality risk in PE patients and improves the decision-making process for optimal therapy. A new question, whether AT substitution would make a better survival, arises. So far, there are no published studies with AT substitution in patients with PE without known hereditary AT deficiency. Substitution with recombinant AT was also reported in septic conditions with disseminated intravascular coagulation [31]. The effectiveness was debatable, but the safety profile was encouraging because no significant increase in major bleeding was reported.

Limitations of the study

All patients received an intravenous bolus of unfractionated heparin immediately prior to admission. Taking a blood sample before receiving heparin was virtually impossible, as this anticoagulant was generally given immediately before admission to the intensive care unit since it is recommended not to wait with the initial heparin bolus while a diagnosis was made. The results of a recently published study show that the same intravenous bolus dose of unfractionated heparin in patients with acute myocardial infarction has a significantly smaller effect on AT activity compared to patients with PE in this study [32]. That is evidence that in addition to the known effect of heparin, massive thrombosis has an additional effect on AT activity.

It is unknown whether some patients had congenital AT deficiency, but this is a very rare thrombophilia and would not affect the overall outcome.

Conclusion

PE patients with low AT activity measured on hospital admission have increased 30-day mortality risk. The measurement of AT activity in the early phase of PE could be used in everyday clinical practice when assessing mortality risk prior to the decision for optimal therapy.

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Is cystatin C a good predictor of acute kidney injury after elective aortic surgery?

Da li je cistatin C dobar prediktor akutne bubrežne slabosti nastale posle elektivne operacije aorte?

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Abstract

Background/Aim. Acute kidney injury (AKI) is a frequent and serious complication after aortic surgery, which increases the length of hospital stay, costs, morbidity, and mortality. The aim of the study was to investigate the incidence of AKI and the most important preoperative and intraoperative predictive factors for AKI 72 hrs after elective infrarenal aortic surgery (IAS). **Methods.** This prospective observational study was performed at the Clinic of Anesthesia, Intensive Care and Pain Therapy, University Clinical Center of Vojvodina (UCCV), from October 2017 to April 2019. It included 140 adult patients who underwent an elective IAS. The occurrence of AKI was noted according to the Acute Kidney Injury Network (AKIN) criteria. A multivariate logistic regression model was used for potential predictive factors. **Results.** The incidence of AKI after the elective IAS at the Clinic of Anesthesia, Intensive Care and Pain Therapy, UCCV, was 28.56%. According to the receiver operating characteristic (ROC) curve analysis, the cut-off value of cystatin C serum concentration of 1.14 mg/L had the highest sensitivity (82.5%) and specificity (76%) in the differentiation of patients who will develop AKI. The final model contained the following variables: the presence of chronic kidney disease, the preoperative serum concentration of cystatin C > 1.14 mg/L, the application of colloid solutions in volume > 500 mL during the operation, and the total intravascular fluid replacement volume > 59 mL/kg in the intraoperative period. **Conclusion.** The incidence of AKI at the Clinic of Anesthesia, Intensive Care and Pain Therapy, UCCV, is somewhat higher compared to the literature data. A presurgical value of cystatin C above 1.14 mg/L is a good predictor of AKI after the elective IAS.

Key words:
aorta; cystatin c; elective surgical procedures; kidney failure, acute; prognosis; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Akutno bubrežno oštećenje (ABO) je česta i ozbiljna komplikacija koja produžava i poskupljuje bolničko lečenje i povećava morbiditet i mortalitet bolesnika nakon hirurške rekonstrukcije abdominalne aorte. Cilj rada bio je da se utvrde incidenca ABO i najznačajniji preoperativni i intraoperativni prediktivni faktori od nastanka ABO 72 sata nakon elektivnih operacija na infrarenalnom segmentu aorte (ISA). **Metode.** Na Klinici za anesteziju, intenzivnu terapiju i terapiju bola Kliničkog centra Vojvodine (KCV) sprovedeno je prospektivno opservaciono istraživanje od oktobra 2017. do aprila 2019. godine. U istraživanje je bilo uključeno 140 bolesnika koji su bili podvrgnuti elektivnom operativnom zahvatu na ISA. Nastanak ABO je potvrđivan na osnovu kriterijuma *Acute Kidney Injury Network* (AKIN) klasifikacionog sistema. Za dobijanje modela predikcije primenjena je multivarijantna logistička regresija. **Rezultati.** Incidenca ABO nakon elektivnih operacija na ISA na Klinici za anesteziju, intenzivnu terapiju i terapiju bola KCV iznosila je 28,56%. Prema analizi *receiver operating characteristic* (ROC) krive, granična vrednost koncentracije cistatina C od 1,14 mg/L imala je najvišu senzitivnost (82,5%) i specifičnost (76%) u diferenciranju bolesnika koji će razviti ABO. Finalni model predikcije ABO nakon elektivnih operacija na ISA sadržao je sledeće faktore: prisustvo hronične bubrežne slabosti, preoperativnu koncentraciju cistatina u serumu C > 1,14 mg/L, primenu koloida u volumenu > 500 mL u toku operacije i ukupni volumen nadoknade u intraoperativnom periodu > 59 mL/kg. **Zaključak.** Incidenca ABO nakon elektivnih operacija na ISA na Klinici za anesteziju, intenzivnu terapiju i terapiju bola KCV je nešto viša u odnosu na podatke iz literature. Preoperativna vrednost cistatina C iznad 1,14 mg/L je dobar prediktor ABO nakon elektivnih operacija na ISA.

Ključne reči:
aorta; cistatin c; hirurgija, elektivna, procedure; bubreg, akutna insuficijencija; prognoza; senzitivnost i specifičnost.

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Introduction

Acute kidney injury (AKI) is a common and severe complication after surgical reconstruction of the abdominal aorta. It prolongs and increases the cost of hospital stay and increases the morbidity and mortality of patients [1, 2]. Earlier diagnosis, monitoring, and earlier initiation of renal replacement therapy (RRT) significantly reduces patient mortality [3, 4].

Due to many AKI definitions, comparing the results of different studies is difficult. The incidence of AKI after elective abdominal aortic aneurysm (AAA) surgery varies from 1–28% depending on the study and the criteria used. AKI is an independent predictor of mortality [5].

Many preoperative and intraoperative factors can influence AKI's occurrence after abdominal aortic surgery. Therefore, it is crucial to understand AKI's risk and the tendency to alter perioperative approaches to prevent or minimize AKI [6].

One of the most important factors for the onset and outcomes of AKI is the patient's renal reserve prior to surgery. Preoperative chronic kidney disease (CKD), measured using serum creatinine (SCr), is associated with a higher risk of AKI following aortic surgery [7, 8].

Cystatin C (CyC) is a 13-kD cysteine protease inhibitor synthesized in all nucleated cells at a steady state. It is freely filtered by the glomerulus, not secreted by renal tubules, and completely metabolized at the level of the renal tubules. These properties have made it an attractive marker of the glomerular filtration rate (GFR) in CKD [9–11].

Factors such as surgical trauma and hemodynamic and humoral changes during and after aortic cross-clamping have been shown to induce ischemia-reperfusion changes and systemic inflammatory response syndrome (SIRS) after aortic surgery [5, 12–13].

Fewer studies about AKI after elective surgery on the infrarenal segment of the aorta have been conducted worldwide.

The aim of this study was to investigate the incidence of AKI and the most essential preoperative and intraoperative predictive factors for AKI.

Methods

This prospective observational study was performed at the Clinic of Anesthesia, Intensive Care and Pain Therapy, University Clinical Center of Vojvodina (UCCV), for 18 months, from October 2017 to April 2019. The study was approved by the Ethics Committee of the UCCV (00-15/34, from February 02, 2017), and all the subjects signed informed consent. The study sample included 140 adult patients who underwent elective infrarenal aortic surgery, with the American Society of Anesthesiologists (ASA) physical status scores of I-III, without CKD or with CKD stage 1 or stage 2. Patients who had to be reoperated and who developed sepsis in the postoperative period of 72 hrs were excluded from the study.

Potential predictive factors, such as patient history, anesthesia lists, daily therapeutic lists, vital parameters, and laboratory values lists, were identified from the medical records. The occurrence of AKI was noted according to the Acute Kidney Injury Network (AKIN) criteria.

During the postoperative 72-hour treatment period, attention was paid to the time and degree of AKI onset and many other significant parameters during the treatment of the patient.

Preoperative period

During the preanesthetic visit, the patients recruited to the study signed informed consent. Relevant anamnestic data were taken, along with necessary medical history and laboratory data obtained by standard measurements and recorded in the research protocol. The following data were obtained: age, gender, diagnosis, body mass, body height, body mass index (BMI), ASA patient status, chronic diseases [the presence of CKD and its stage; cardiovascular diseases (CVD): documented hypertension, ischemic heart disease, myocardial infarction or valvular heart disease; documented diabetes mellitus, type 1 or 2 (fasting plasma glucose $\geq$ 7.0 mmol/L or venous plasma glucose 2 hrs after ingestion of 75 g oral glucose load $\geq$ 11.1 mmol/L); documented chronic obstructive pulmonary diseases (COPD): chronic bronchitis or emphysema (abnormalities in the small airways of the lung lead to limitation of airflow in and out of the lungs); documented previous cerebrovascular stroke, ischemic or hemorrhagic (a focal or global disorder of brain function that occurs suddenly, and is a consequence of cerebral circulation disorder or a condition in which blood flow is insufficient to meet the metabolic needs of neurons for oxygen and glucose); documented hypothyroidism (which is proven by low level of serum thyroxine and high level of thyroid-stimulating hormone); chronic drug therapy (with emphasis on nephrotoxic drugs: nonsteroidal anti-inflammatory drugs, angiotensin converting enzyme inhibitors, angiotensin receptor inhibitors, diuretics, proton pump inhibitors, H2 inhibitors); pernicious habits (smoking, alcoholism); information on common values of arterial blood pressure; laboratory values of blood urea nitrogen (BUN), creatinine, CyC, glucose in blood].

Creatinine clearance (ClCr) was calculated using the Cockcroft-Gault formula: ClCr, mL/min = [(140 – age, years) × body weight, kg] / 0.814 × SCr, μmol/L × 0.85 (for female) and GFR by plasma concentration of CyC, GFR CyC, mL/min (CKD-EPI CyC Equation-2012).

Intraoperative period

All patients received general balanced endotracheal anesthesia, with standard monitoring for these types of operations: continuous electrocardiography, noninvasive measurement of arterial blood pressure, invasive measurement of arterial blood pressure (arterial cannula placed in the radial artery), measurement of central venous pressure (central venous catheter placed via internal jugular or subclavian vein), pulse oximetry, capnometry, and measurement of urine output. During the surgery, controlled mechanical ventilation with positive end-expiratory pressure (PEEP) of 3–5 mmHg

was performed with FiO_2 50% to achieve normocapnia. The patients were actively warmed during the surgery. The main aim was to maintain hemodynamic parameters within 20% of their basal values.

The following parameters were recorded in the research protocol: the duration of operation, the duration of aortic cross-clamping, blood loss volume, hourly and total diuresis, the amount and type of intravenous fluid given, the amount of autologous blood volume given, the amount and type of heterologous blood products given, the duration of episodes of hypotension when median arterial pressure (MAP) < 65 mmHg, use of vasopressors, diuretics, the minimal value of central venous pressure, PEEP value, the laboratory data from gas analysis of arterial and central vein blood [blood lactate level, acid-base status: pH, base excess (BE), blood glucose concentration, the saturation of central venous blood].

Postoperative period

After the surgery, the patients were sedated, intubated, and placed in the intensive care unit according to the procedure for transporting patients from the operating block and treated according to all the principles of intensive care. Within 72 hrs after surgery, attention was paid to the course of treatment, the time of onset of AKI, its degree, and the eventual initiation of RRT. The following parameters were monitored daily: hourly and total diuresis, the use of vasoactive drugs, the use of diuretics, the number of hrs when arterial pressure was lower than normal preoperative pressure, number of hrs when MAP < 65 mmHg, laboratory data: BUN, blood creatinine, CyC, and glucose concentration, the concentration of electrolytes in blood (sodium, potassium, chlorine, magnesium), blood lactate level, acid-base status: pH, base excess (BE).

Acute Physiology and Chronic Health Evaluation (APACHE) II score on the first day and the Sequential Organ Failure Assessment (SOFA) score, ClCr, and GFR using plasma CyC (GFR CyC) concentrations were calculated for all three days.

BN ProSpect plasma protein analyzer – Siemens Healthineers Global, was used to measure CyC concentration (the method was nephelometric immunoassay: particle enhanced nephelometric immunoassay – PENIA), while serum creatinine was measured by Jaffe reaction.

Statistical analysis

Arithmetic means with standard deviation or median with the range including minimum and maximum values were used to describe the continuous numerical characteristics as a measure of central tendency. The estimation of the normality of continuous variables distribution was performed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Categorical variables were described using absolute and relative numbers.

Depending on the type and the normality of the distribution of variables, the differences between the studied groups were compared using appropriate parametric and non-parametric tests.

For the AKI development prediction model, all independent factors (variables) of preoperative and intraoperative status whose statistical significance was $p < 0.05$ were analyzed. To improve the clinical utility of the results obtained, continuous variables were transformed into dichotomous ones based on the maximum sum of sensitivity and specificity estimated by the receiver operating characteristic (ROC) analysis of curves and cut-off values obtained. Then, a univariate analysis was performed to check the statistical significance and to calculate the odds ratio (OR) and 95% confidence intervals (CIs). The collinearity of the examined variables was also verified using Pearson's correlation test and collinearity test. For the formation of the complete logistic regression model, all those variables were taken, which, after univariate analysis, were $p < 0.05$ and the value of probability coefficient greater than 2.

Logistic regression results are represented by a beta coefficient (Beta), standard error (SE), *p*-value, and probability coefficient with 95% CI. The ROC curve, the positive and negative values, and the sensitivity and specificity of the complete model were also calculated. The significant predictors of AKI development were those variables that reached $p < 0.05$.

IBM SPSS version 21 (Chicago, Illinois) was used for the statistical analysis. The results were presented in Tables and Figures; statistical significance was set at the *p*-value of less than 0.05.

Results

The total number of enrolled patients was 174. Preoperatively, 5 patients were excluded – two patients with CKD stage 3, two patients with ASA status IV, and one patient who refused to participate in this study. Intraoperatively, one patient was excluded because of the need for suprarenal cross-clamping. Postoperatively, 2 patients were excluded because of the need for re-operation and 26 because of missing data and noncompliance with the research protocol.

The total number of patients who completed the study was 140. Among them, 40 (28.56%) developed AKI; 32 (80%) of them developed AKIN 1, 6 (15%) AKIN 2, and only 2 (5%) AKIN 3 stage of the disease.

The mean age of the patients was 67.17 ± 6.53 years. Most patients were male (82.14%), with ASA status III (95.71%) and a mean BMI of 26.97 ± 5.28 kg/m^2. One hundred and thirteen (92.86%) patients had high blood pressure, 35 (25%) had COPD, 20 (14.28%) were diabetics, 15 (10.71%) had CVD, and 15 (10.71%) had CKD. One hundred and eleven (79.29%) patients underwent surgery for a diagnosed AAA and 29 (20.71%) for aortic occlusive disease (Leriche syndrome).

Univariate analysis revealed the differences at the $p < 0.05$ level between cases and controls in the sample for age, diagnosis, CKD, BUN, SCr, ClCr, CyC, GFR using plasma CyC, the use of acetylsalicylic acid or other antiplatelet drugs, oliguria in intraoperative period, the value of BE in-

traoperatively, colloid volume greater than 500 mL, the use of fresh frozen plasma (FFP) and resuspended erythrocytes (RE) intraoperatively, the total volume of fluid given intraoperatively greater than 59 mL/kg (Table 1).

Logistic regression was conducted to evaluate the impact of multiple independent factors on the likelihood of developing AKI. The model contained 11 independent variables: age, CKD, BUN, SCr, CyC, other antiplatelet drugs, oliguria, colloid volume, FFP and RE, and total fluid replacement volume (Table 2).

The whole model (with all predictors) was statistically significant, $\chi^2 = 74.753$, $p < 0.001$ (DF = 11, n = 140), which shows that the model distinguishes between the patients who developed AKI and those who did not. The model explains between 41.4% (R-squared Cox and Snell) and 59.3% (R-squared Nagelkerke) of the variance in AKI status and accurately classifies 87.9% of cases. As seen in the Table 2, only four independent variables made a unique statistically significant contribution to the model (CKD, CyC, colloid volume, and total fluid volume).

The strongest predictor of AKI development was a CyC concentration above 1.14 mg/L, with a probability ratio of 17.811. That shows that the patients who preoperatively have a CyC concentration above 1.14 mg/L are over 17 times more likely to develop AKI, with all other elements in the model being equal. A CKD with a probability factor of 8.569 is in second place, suggesting that patients with CKD are 8 times more likely to develop AKI, with all other factors in the model being equal. The OR for the colloid volume used intraoperatively and for total fluid volume is over 4. In other words, patients treated with a colloid volume greater than 500 mL or a total fluid volume greater than 59 were 4 times more likely to develop AKI, with all other factors in the model being equal. The classification table obtained from the logistic regression shows that this model correctly classifies 72.5% of the respondents who developed AKI (sensitivity). Correspondingly, this model accurately identified 94% of individuals who did not develop AKI (specificity). The positive predictive value was 82.9%, and the negative predictive value was 89.5%.

The ROC area under the curve (AUC) of the complete predictor model was 0.932, and the 95% CI was 0.889–0.971 (Figure 1, Table 3).

Table 1

The results of univariant logistic regression

Variable	Beta	SE	*p*-value	OR (95% CI)
Age (> 65 years)	1.471	0.462	0.001	4.352 (1.760–10.757)
Leriche syndrome	-1.099	0.575	0.048	0.333 (0.108–1.030)
CKD	2.209	0.621	< 0.001	9.103 (2.695–30.755)
BUN (> 6.15 mmol/L)	1.466	0.404	< 0.001	4.333 (1.964–9.561)
SCr (> 80 μmol/L)	1.054	0.394	0.007	2.868 (1.326–6.205)
ClCr (< 79 mL/min)	-1.439	0.399	< 0.001	0.237 (0.108–0.518)
Cystatin C (> 1.14 mg/L)	2.652	0.463	< 0.001	14.182 (5.721–35.155)
GFR (< 63 ml/min)	-2.351	0.469	< 0.001	0.095 (0.038–0.239)
Acetylsalicylic acid	-0.853	0.382	0.026	0.426 (0.201–0.902)
Other antiplatelet drugs	1.365	0.578	0.018	3.917 (1.263–12.148)
Oliguria	1.023	0.469	0.029	2.782 (1.109–6.796)
BE (mmol/L)	-0.161	0.078	0.039	0.851 (0.731–0.992)
Colloid (> 500 mL)	0.999	0.392	0.011	2.714 (1.259–5.851)
FFP	1.084	0.444	0.015	2.958 (1.239–7.059)
RE	1.466	0.474	0.002	4.333 (1.710–10.981)
Volume (> 59 mL/kg)	1.206	0.388	0.002	3.339 (1.559–7.149)

SE – standard error; OR – odds ratio; 95% CI – confidence interval; CKD – chronic kidney disease; BUN – blood urea nitrogen; SCr – serum creatinine; ClCr – creatinine clearance; GFR – glomerular filtration rate; BE – base excess; FFP – fresh frozen plasma; RE – resuspended erythrocytes.

Table 2

Results of multivariant logistic regression

Variable	Beta	SE	*p*-value	OR (95% CI)
Age (> 65 years)	0.565	0.676	0.403	0.569 (0.151–2.138)
CKD	2.148	0.922	0.020	8.569 (1.407–52.181)
Urea (> 6.15 mmol/L)	0.477	0.600	0.426	1.612 (0.497–5.223)
SCr (> 80 μmol/L)	0.156	0.655	0.812	1.169 (0.324–4.219)
Cystatin C (> 1.14 mg/L)	3.376	0.833	< 0.001	17.811 (4.581–69.252)
Other antiplatelet drugs	1.554	0.945	0.100	4.730 (0.742–30.166)
Oliguria	1.172	0.838	0.162	3.229 (0.625–16.690)
Colloid (> 500 mL)	1.489	0.705	0.035	4.432 (1.113–17.654)
FFP	0.964	0.717	0.179	2.622 (0.643–10.691)
RE	-0.396	0.792	0.617	0.673 (0.143–3.180)
Volume (> 59 mL/kg)	1.453	0.687	0.034	4.274 (1.112–16.424)

SE – standard error; OR – odd ratio; 95% CI – confidence interval; CKD – chronic kidney disease; SCr – serum creatinine; FFP – fresh frozen plasma; RE – resuspended erythrocytes.

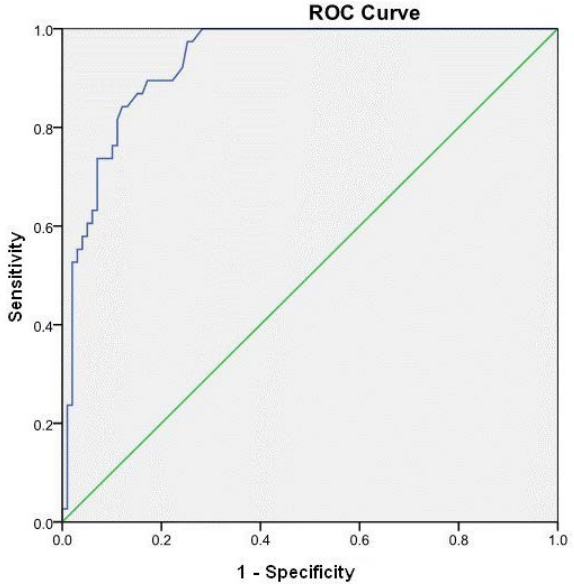

Fig. 1 – A receiver operating characteristic (ROC) curve of the complete logistic regression model.

Table 3

A receiver operating characteristic (ROC) curve analysis

AUC	SE	*p*-value	95% CI
0.932	0.022	< 0.001	0.889–0.971

AUC – area under the curve; SE – standard error; CI – confidence interval.

Discussion

Our results show that the incidence of AKI after elective aortic surgery is 28.56%, which is slightly higher compared to the literature data. The reason may be the use of different diagnostic criteria for AKI in our and other studies. We used a more sensitive AKIN classification, where the definition of AKI is not based on an increase in serum creatinine relative to the individual baseline value as in the RIFLE classification but on the initially measured value, and the change is observed within 48 hrs [14]. The incidence of AKI after elective AAA surgery varies from 1–28% depending on the study and the applied criteria [5, 15–17]. Bang et al. [18] showed in their study that AKI, according to AKIN criteria, was developed in 18.5% of patients and according to RIFLE criteria in 12.4% of patients after AAA surgery. In a study that included patients with AAA surgeries – endovascular, open, ruptured, and unruptured – the incidence of AKI in open surgery for unruptured aneurysms was 26.2%, while in ruptured aneurysms it was 48.1% [19]. Few papers investigated the incidence of AKI after aortic occlusive disease surgery. In a study evaluating AKI in elective open aortic surgery, the incidence was 22%, using RIFLE diagnostic criteria [5]. The average age of the examined patients in our study was 67.17 years (standard deviation – SD, 6.53), which is in accordance with the literature data. As age increases, so does the prevalence of the aneurysmal and aortic disease. The prevalence of AAA is about 5% in the population older than 65 years. Some studies have shown that the average age of the patients who underwent AAA surgery was 72 years [20, 21]. Our study included much more men – 115 (82.14%), than women, 25 (17.86%). The ratio between men and women was 4.6: 1, similar to the known literature data, where it is stated that the number of men exceeds the number of women among these patients, and the ratio is 4–6 : 1 [22, 23].

Demographic factors such as age and gender are closely related to the development of postoperative AKI. The occurrence of AKI as a complication in the postoperative period increases with age. The capacity of the kidneys to adapt to hemodynamic changes decreases with age [24]. Further, the renal blood flow and response to vasodilating factors decrease in old age [25]. Numerous studies have shown that the male gender is a risk factor for the development of postoperative AKI [26, 27]. However, in a recent prospective study that evaluated 9,400 patients after cardiac surgery, the female gender was shown to be a significant risk factor for the development of postoperative AKI [28]. Our study showed that there was no statistically significant difference in the distribution of the examined patients by groups according to gender. In the group of patients with AKI, there were 10 (25%) women and 30 (75%) men, while in the group of patients with no AKI, there were 15 (15%) women and 85 (85%) men.

From the examined potential predictors of AKI in the preoperative and intraoperative period, we found a total of 11 factors that had a statistically significant ($p < 0.05$) individual influence (Table 2). After the analysis of the potential risk factors, we obtained a final prediction model consisting of the presence of CKD, preoperative CyC concentration > 1.14 mg/L, intraoperative colloid replacement in a volume > 500 mL, and total intraoperative replacement volume > 59 mL/kg.

The strongest predictor of AKI development was the preoperative CyC concentration above 1.14 mg/L, with an OR of 17.811. That shows that patients with a preoperative CyC concentration above 1.14 mg/L are over 17 times more likely to get AKI, with all other factors equal in the model. CyC is an excellent marker of glomerular filtration rate: it is freely filtered through the glomeruli, completely reabsorbed in the proximal tubules, and not secreted by the renal tubules. It does not have as much interindividual variation and limitation as creatinine, e.g., due to the influence of muscle mass, diet, sex, and tubular secretion. Therefore, CyC is a better marker of GFR than creatinine [29, 30], especially in cases where there is a subclinical increase in creatinine, which is not a criterion for defining renal impairment by current definitions [29, 31, 32]. In our study, we found out that the preoperative value of CyC in the group of patients with AKI was statistically significantly higher, and the strength of the impact was assessed as extremely high. Specifically, the mean CyC concentration in the group of patients who did not receive AKI was 0.96 (SD = 0.27) mg/L, while in the group of patients who received AKI, it was 1.31 (SD = 0.25) mg/L. According to the ROC curve analysis, the concentration limit value for CyC was 1.14 mg/L. For this value, the maximum sensitivity index was 82.5%, and the specificity 76%. The

area under the curve was 0.830, and the 95% CI was 0.761–0.900. Seven (8.4%) out of 83 patients with a CyC concentration less than or equal to 1.14 mg/L developed AKI, and 33 (57.9%) of 57 patients with a CyC concentration greater than 1.14 mg/L developed AKI. According to our previous knowledge, there is no literature data on the examination of the predictive value of the preoperative concentration of CyC in vascular surgery. However, there is plenty of literature on the predictive value of preoperative CyC concentration in cardiac surgery [33, 34]. The reason could be the highest incidence of AKI after cardiac surgery. The literature data are in agreement with our results. A Translational Research Investigating Biomarker Endpoints in Acute Kidney Injury (TRIBE-AKI) study in 1,147 cardiac surgery patients demonstrated that preoperative CyC concentration was a better predictor of AKI after cardiac surgery than preoperative SCr and ClCr [35]. Turki et al. [36] confirmed in their research that preoperative, baseline, serum CyC value is a good predictor of AKI after cardiac surgery. All the above suggests that it would be rational to find out the preoperative value of CyC in risky surgery because it has been proven to be a good predictor of postoperative AKI.

In second place is the presence of CKD with an OR of 8.569. That suggests that patients with CKD are 8 times more likely to develop AKI, with all other factors equal in the model. CKD unequivocally increases the risk of acute renal damage, which is in line with numerous literature data [19, 37]. CKD significantly contributes to the formation of AKI and *vice versa* [38]. In our study, we included the patient with mild CKD (1st and 2nd degree) and excluded those with CKD 3rd, 4th, and 5th degrees. Of all the associated diseases, only a statistically significant difference between the examined groups was found for the presence of mild chronic renal failure. A statistically significantly higher number of subjects with CKD was observed in the group of patients with AKI. Of the 40 patients who developed AKI, 11 (27.5%) had a history of stage 1 or 2 CKD, and of the 100 who did not develop AKI, only 4 (4%) had CKD.

The probability ratio for intraoperative colloid replacement in a volume > 500 mL and the total intraoperative replacement volume > 59 mL/kg is over 4. That means that patients who received > 500 mL of colloidal solutions intraoperatively and who received a total replacement volume greater than 59 mL/kg are 4 times more likely to develop AKI. It was observed that in the group of patients who received a larger volume of colloidal solutions (from 501 mL to 1,000 mL), a statistically significantly higher number of patients developed AKI, 19 (47.5%), compared to patients who did not develop AKI, 25 (25%). This result agrees with previous research on the effect of hydroxyethyl starch (HES) solution on renal function. For decades, there has been a debate about intravenous fluid therapy on whether colloids or crystalloids are better or more harmful. There is no doubt that it will continue in the future. However, the detrimental effect of synthetic colloidal solutions on the kidneys is known [39]. Newer solutions have a lower molecular weight and a lower degree of substitution, the two changes that reduce accumulation and toxicity. We used 6% HES solutions with 130 kDa and a degree of substitution of 0.42 in a physiologically balanced solution. Many studies conducted to investigate the effect of HES solution on renal function in sepsis have shown that the use of HES solution is an independent risk factor for the development of AKI in severe sepsis [40] and that the usage of HES increases the need for RRT. The greater the cumulative dose of colloid, the greater the adverse effect [41]. Furthermore, a meta-analysis of ten studies examining this topic in critically ill septic patients showed that HES administration was associated with higher 90-day mortality, an increased risk of developing AKI, and a higher need for RRT [42]. The detrimental effect of HES solution on the kidneys and among surgical patients in the perioperative period has also been proven [43]. Intravenous fluid therapy is widely used for both the prevention and treatment of AKI in the belief that the most common cause of AKI is the prerenal component. On the other hand, the harmful effects of a fluid overdose can be emphasized in situations such as severe sepsis, major surgery, and trauma, which are predisposing factors for the development of AKI [44, 45].

Intravenous fluid therapy is the most exploited therapy for patients at risk of AKI. However, the harmful consequences of fluid therapy are being increasingly recognized. In fact, adequate fluid therapy in patients with AKI is the key to the treatment. The administration of crystalloid solutions increases the intravascular compartment, but over time it it and is distributed in the interstitial compartment. Renal interstitial edema worsens renal function. Because the kidney is an encapsulated organ, when congestion occurs, venous pressure also increases, along with intracapsular pressure, which all leads to a decrease in renal blood flow and GFR. Observational studies in critically ill patients have found a link between positive fluid balance and AKI [46, 47]. Moreover, excessive fluid intake results in visceral edema, which is a risk factor for the occurrence of intraabdominal hypertension. Elevated intraabdominal pressure increases renal venous pressure and decreases renal blood flow and GFR [48]. A conservative fluid replacement strategy is recommended with the goal of neutral and a negative fluid balance when hemodynamic stability is achieved. In patients with AKI, this strategy involves the earlier use of RRT in the initial phase of treatment, when a more liberal fluid replacement strategy is used [49]. In general, an individualized approach to each patient and goal-directed fluid therapy (GDT) are emphasized to avoid iatrogenic harmful fluid therapy [50].

The classification table of logistic regression shows that the final model accurately classified 72.5% of respondents who developed AKI (sensitivity). In addition, this model accurately identified 94% of individuals who did not develop AKI (specificity). The positive predictive value was 82.9%, and the negative predictive value was 89.5%. The ROC AUC of the complete predictor model was 0.932, and the 95% CI was 0.889–0.971. A Chinese study in 2017 on risk factors after AAA surgery showed a predictive score for AKI (WCR-DA score), which contains the following elements for elective surgeries: smoking, blood loss > 1 L, and antihyperten-

sive therapy. A score of 0 has a predicted risk of developing AKI of 2%, while a maximum score of 4 carries a risk of 78% [51]. A recent Korean study obtained its predictive model for the development of AKI after aortic surgery, which includes age > 60 years, decreased preoperative GFR, preoperatively reduced left ventricular systolic function, prolonged operative time, intraoperative oliguria, and intraoperative furosemide therapy [52]. In an Italian study comparing the incidence of AKI after endovascular and open elective operations on the infrarenal aorta, the development of AKI was significantly associated with smoking, hypertension, chronic renal failure, open aortic surgery, and arrhythmias [53].

Conclusion

Levels of preoperative CyC above 1.14 mg/L are a good predictor of AKI in patients undergoing elective infrarenal aortic surgery. These findings support the use of routine preoperative CyC measurements with all other important predictors.

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Anti-SARS-CoV-2 antibody responses in convalescent plasma donors with varying clinical manifestation severity of COVID-19

Anti-SARS-CoV-2 antitela kod rekonvalescentnih davalaca plazme sa različitom težinom kliničke slike COVID-19

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Abstract

Background/Aim. Plasma containing a high titer of anti-SARS-CoV-2 antibodies, donated from individuals who recovered from COVID-19, has the potential to be used as initial therapy for patients who have been infected (passive immunization). It is a challenge to find suitable donors. The aim of the study was to successively monitor antibody titer in donations and to investigate the correlation between antibody titer and the severity of the clinical manifestations. **Methods.** The retrospective study was conducted from May 1 to October 31, 2020, at the Blood Transfusion Institute of Vojvodina. Donors had to meet certain criteria for inclusion in the study: proven SARS-CoV-2 infection, detected SARS-CoV-2 antibodies in the serum/plasma, fulfillment of general criteria for performing plasmapheresis, and adequate laboratory findings. **Results.** During the study, 651 apheresis plasma units were collected and divided into two equal doses. Plasma was donated by 311 COVID-19 convalescents, including 208 (66.9%) men and 103 (33.1%) women. There were 15 (4.8%) plasma donors with asymptomatic infection, 235 (75. 6%) with a mild form of illness, 45 (14.5%) with a moderate form of illness, 16 (5.1%) with a severe form of illness, and none with a critical form of illness. Anti-SARS-CoV-2 IgG antibodies were present in the plasma of donors for more than 6 months after the disease. Plasma donors with a more severe clinical manifestation of COVID-19 had stable antibody levels for a longer period. However, the Pearson correlation of clinical severity and antibody titer did not confirm a statistically significant correlation between the variables. **Conclusion.** Anti-SARS-CoV-2 antibodies were present in the sample of recovered patients, plasma donors, for more than 6 months after the disease. Even though no statistically significant correlation was found between the anti-SARS-CoV-2 antibody titer and the clinical severity of COVID-19, in patients with a more severe clinical manifestations of the disease, stable antibody levels were maintained for a longer period.

Key words:
antibody formation; blood donors; COVID-19 serotherapy; immunization, passive; plasma.

Apstrakt

Uvod/Cilj. Plazma koja sadrži visok titar antitela na SARS-CoV-2, donirana od osoba koje su se oporavile od COVID-19, ima potencijal da se koristi kao inicijalna terapija kod obolelih u vidu pasivne imunizacije. Poseban izazov predstavlja izbor odgovarajućih davalaca plazme. Cilj rada bio je sukcesivno praćenje titra antitela u donacijama plazme i ispitivanje korelacije između titra antitela i težine kliničke slike koju su davaoci imali tokom bolesti. **Metode.** Retrospektivna studija sprovedena je od 1. maja do 31. oktobra 2020. godine na Institutu za transfuziju krvi Vojvodine. Davaoci su morali da ispune određene kriterijume za uključivanje u studiju: dokazana infekcija SARS-CoV-2, prisutna antitela na SARS-CoV-2 u serumu/plazmi, ispunjavanje opštih kriterijuma za izvođenje plazmafereze i referentne vrednosti laboratorijskih nalaza. **Rezultati.** Tokom studije prikupljeno je 651 jedinica aferezne plazme podeljenih u dve jednake doze. Plazmu je doniralo 311 COVID-19 rekonvalescenata, uključujući 208 (66,9%) muškaraca i 103 (33,1%) žena. Davalaca plazme sa asimptomatskom infekcijom bilo je 15 (4,8%), sa blagim oblikom bolesti 235 (75,6%), sa umereno teškim oblikom bolesti 45 (14,5%), sa teškim oblikom bolesti 16 (5,1%).

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Osobe sa kritičnim oblikom bolesti nisu donirale plazmu. Antitela klase IgG na SARS-CoV-2 bila su prisutna kod davalaca plazme više od šest meseci nakon bolesti, pri čemu su davaoci plazme koji su imali težu kliničku sliku COVID-19 imali stabilne vrednosti antitela tokom dužeg vremenskog perioda. Pearson-ova korelacija težine kliničke slike i titra antitela nije potvrdila njihovu statistički značajnu povezanost. **Zaključak.** Antitela klase IgG na SARS-CoV-2 bila su prisutna kod davalaca plazme više od šest meseci nakon bolesti. Iako nije nađena statistički značajna korelacija između titra antitela klase IgG na SARS-CoV-2 i težine kliničke slike COVID-19, utvrđeno je da se kod bolesnika koji su imali težu kliničku sliku bolesti, stabilan titar antitela održavao tokom dužeg vremenskog perioda.

Ključne reči:
antitela, stvaranje; krv, davaoci; COVID-19 seroterapija; imunizacija, pasivna; plazma.

Introduction

The most recently discovered severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) that causes coronavirus disease (COVID-19) appeared in Wuhan, China, in late 2019 [1]. The outbreak of the disease very quickly grew into a pandemic that was officially declared by the World Health Organization (WHO) on March 11, 2020 [2]. The virus, which belongs to the Coronavirus family with single-stranded ribonucleic acid (RNA), penetrates cells through the angiotensin-converting enzyme 2 (ACE2) receptor, which can be found on the cell surface of the heart, lungs, kidneys, gastrointestinal tract, and, as proven most important, on alveolar epithelial cells. It spreads from person to person by droplets, and most patients have a clinical presentation with mild symptoms. However, there may be a sudden deterioration of the patient's health, ranging from mild clinical picture to severe pneumonia with accompanying complications such as acute respiratory syndrome, sepsis, massive thromboembolism, hypercoagulability, and renal failure. Studies show that about 14% of patients with pneumonia develop a severe clinical presentation with a possible fatal outcome [3].

According to the WHO, up to and including January 15, 2021, over 223 countries were affected by the pandemic, and the virus infected 91,816,091 people, of whom 1,986,871 were fatalities (2.16% mortality rate) [4]. Apart from the fact that there is no specific therapy for COVID-19, the vaccine, the administration of which began in late 2020, is not expected to be available in sufficient quantities and in a short period of time in all world countries. The WHO and The Food and Drug Administration (FDA) issued a recommendation for the clinical trial of using convalescent plasma in patients who recovered from COVID-19 in 2020 [5, 6]. That is not the first recommendation for using convalescent whole blood or plasma in patients who have been infected. So far, the WHO has recommended a clinical trial in several cases: human influenza A (H1N1) in 2009, the Ebola epidemic in West Africa in 2014, Middle East respiratory syndrome (MERS) in 2015, and avian influenza A (H5N1) in 2019 [7].

Passive immunization with plasma containing a high titer of anti-SARS-CoV-2 antibodies has the potential to be used as an initial therapy. In addition, previous research shows that its application is most effective in the first three days after diagnosis or hospitalization of the patient [8].

Regardless of examining clinical parameters relevant to the timely administration of plasma, finding convalescent plasma donors with therapeutic potential poses a particular challenge. Studies show that the amount of antibodies that neutralize viral activity in the serum varies drastically among patients. A Chinese study described that 6% of patients did not produce detectable antibodies and that 30% had a very low titer [9].

The first apheresis procedure for the collection of convalescent COVID-19 plasma (CCP) for therapeutic purposes in the territory of Vojvodina was performed on May 1, 2020, as a part of the National Program for the CCP collection in Serbia. The study gives the qualifications which need to be fulfilled by donors in order to be included in the National Program, along with donor demographic characteristics. The main goal of the study was to successively monitor antibody values in donated plasma samples and investigate the correlation between antibody index and the severity of the clinical manifestations of COVID-19.

Methods

Study design

The retrospective study was conducted from May 1 to October 31, 2020, at the Blood Transfusion Institute of Vojvodina (BTIV), Novi Sad, Serbia. Data collected during the preparation of potential donors and during apheresis procedures were recorded in specially formed Registers of anti-COVID-19 plasma donors and the BTIV information system from where they were used for analysis.

The study was approved by the Ethic Committee of the BTIV with approval number 01–809/20 on November 16, 2020.

Donor inclusion criteria

Criteria for inclusion of patients who recovered from COVID-19 (potential donors) in the plasmapheresis procedure were as follows: proven SARS-CoV-2 infection either by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) from a nasopharyngeal swab specimen or serologically detected SARS-CoV-2 IgG antibodies (chemiluminescent immunoassay or enzyme-linked immunosorbent assay) in the serum/plasma of a potential donor – findings from any accredited laboratory were taken into consideration; more than 14 days have passed since the withdrawal of symptoms; there are no signs of acute infection; fulfillment of general criteria for performing plasmapheresis checked through questions in the questionnaire for donors and physician ex-

amination, and, thus, plasma donors can be persons between 18–60 years of age, weighing more than 60 kg, without comorbidities which are permanent contraindications for blood donation; appropriate vascular access for plasmapheresis procedure.

Laboratory testing

On the first arrival and after three months from the start of plasma administration, the following tests were performed: a rapid chromatographic test for antibodies [Innovita 2019-nCoV IgM/IgG Ab Test (Colloidal Gold), Innovita (Tangshan) Biological Technology CO., LTD, Hebei, China]; complete blood count with white blood cell count; biochemical analyses including total proteins, albumin, immunoglobulins (IgG, IgM, IgA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total and direct bilirubin, urea, creatinine, C-reactive protein (CRP) test; coagulation status [prothrombin time (PT) and international normalized ratio (INR), activated partial thromboplastin time (aPTT)]; donors with deviations from the normal values of these analyses were temporarily excluded from the procedure until their findings normalized; screening for anti-HLA (human leukocyte antigen) class I and II antibodies was performed for the donors with a prior history of pregnancy or transfusion; positive-test individuals (anti-HLA I and/or II) were excluded from the apheresis procedure (a total of 11 people) due to the prevention of transfusion-related acute lung injury – TRALI.

At each plasma donation, the following tests were performed: a) ABO/RhD blood group; b) red blood cell antibody screening; c) serological and molecular tests for markers of four transfusion-transmitted pathogens (human immunodeficiency virus, hepatitis B virus, hepatitis C virus, Treponema pallidum).

Donors with positive red blood cell antibody screening and/or serological/molecular tests for markers of transfusion-transmitted infections were permanently excluded from the procedure.

Before each plasma donation, in accordance with National Program for the CCP collection in Serbia, the value of the SARS-CoV-2 IgG antibody index was determined for all plasma donors' samples (Virclia COVID-19 ELISA IgG, Vircell S.L, Granada, Spain). Captured anti-SARS-CoV-2 antibodies were total antibodies to spike (S) glycoprotein and nucleocapsid (N) protein. Only donors with an antibody index > 6 were included in the procedure. Interpretation of the value over 6 was considered positive according to manufacturer protocol and used as appropriate for covid plasma donation.

During the first month of the observed period, plasma donors' antibodies were subsequently determined from archived specimens when serological tests became available. Donors with SARS-CoV-2 IgG antibody values below the cut-off were excluded from the program and are not the subject of research. Plasma with minimum antibody values over 12 was used for therapeutic purposes in the BTIV.

Plasma collection procedure

Plasma was collected by apheresis procedure on automated Haemonetics MCS + separators. The procedure lasted from 30 to 40 minutes, while the amount of plasma taken in the standard procedure was from 500 to 600 mL. Each unit taken was divided into two equal doses. Plasma was frozen within 6–8 hrs of collection and labeled "apheresis anti-COVID-19 fresh frozen plasma – clinical trial" with ABO blood group and laboratory testing for the transfusion-transmitted disease.

Assessment of disease severity

A potential donor with SARS-CoV-2 infection was grouped into the following severity of illness categories: Asymptomatic infection (AI): individuals with no symptoms that are consistent with COVID-19; Mild illness (MI): individuals with any of the following various signs and symptoms of COVID-19 – fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell; Moderate illness (MoI): individuals with shortness of breath, dyspnea, or abnormal chest imaging (showed evidence of lower respiratory tract disease during clinical assessment or imaging, oxygen saturation (SpO2) ≥ 94%); Severe illness (SI): individuals with SpO2 < 94%, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) < 300 mmHg, respiratory frequency > 30 breaths/min, or lung infiltrates > 50%; Critical illness (CI): individuals with respiratory failure, septic shock, and/or multiple organ dysfunction.

Statistical analysis

Data collected were analyzed using the statistical program Minitab 16, Wessa.net Pearson Correlation – Free Statistics Software (Calculator), and Microsoft Excel. Descriptive statistics were conducted for all variables. Data are presented in tables and graphs. Statistical significance was set at $p < 0.05$.

Results

During the study, 651 apheresis plasma units were collected and divided into two equal doses. Over the study period, plasma was donated by 311 COVID-19 convalescents, including 208 (66.88%) men and 103 (33.12%) women. The youngest donor was 18 years old, and the oldest was 60 (mean 38.40). Among donors, the most represented age group was 35–39 (Figure 1).

Donors distribution was as follows: a) by the place of residence: 3 donors from rural areas, 91 from urban settlements, and 217 from the city; b) by the time of falling ill: March 31, April 17, May 14, June 15, July 18, August 15, September 29, October 12; c) by the region of residence: South Bačka 200, West Bačka 9, North Bačka 13, North Banat 5, Central Banat 6, South Banat 28, Srem 44, Belgrade 4, Valjevo 2.

The number of donations during the study period was as follows: May 43, June 79, July 115, August 123, September 150, and October 141. Of the 311 convalescents, 52.7% donated plasma once (n = 164), 18.7% twice (n = 58), 10.9% three times (n = 34), 8.4% four times (n = 26), 4.8% five times (n = 15), 2.6% six times (n = 8) and 1.9% seven times (n = 6). Among donors residing outside the South Bačka district, 60.4% (n = 67) donated plasma only once.

In donors who donated plasma more than three times, it was noticeable that antibody titer values were maintained even after 6 months.

The index values of the serological test for SARS-CoV-2 IgG antibodies in donors ranged from 1.02 to 117.03 (average value 29.54). The distribution of donors with different SARS-CoV-2 IgG antibody index values is shown in Figure 2.

The rapid antibody detection test was positive in 187 (60.13%) and negative in 124 (39.87%) donors. The lowest value of SARS-CoV-2 IgG at which the rapid test was positive was 19.75. The rapid test was positive in all donors with a SARS-CoV-2 IgG index greater than 30.

During the six months of the observed period, donors from all groups with different illness severity criteria donated plasma at different time intervals (from 14 days and up) and a different number of times (from 1 to 7). On the first donation: the value of the index in the donors with MI and AI ranged from 1.03 to 117.03 (lower and upper extremes of a set of data), while in the donors with MoI and SI the values ranged from 3.97 to 102.82; the median was 60 in the donors with MI and AI and 54 in the donors with MoI and SI; the upper quartile (the median of the upper region) and the lower quartile (the median of the lower region) was 88 and 30 in

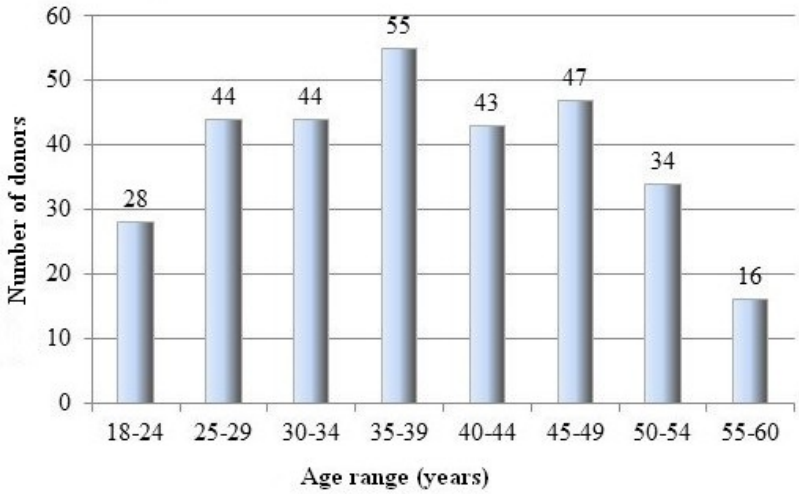


Fig. 1 – Age distribution of convalescent plasma donors.

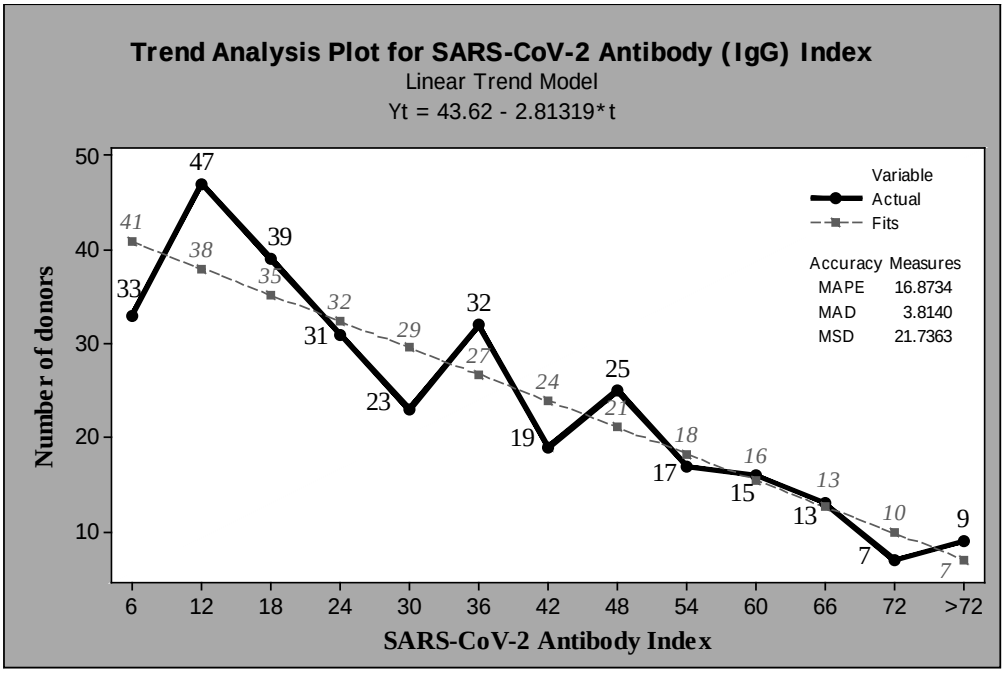


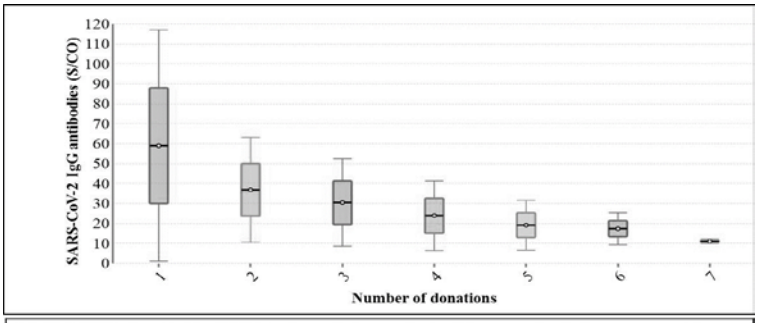
Fig. 2 – Distribution of covalescent plasma donors with different SARS-CoV-2 IgG antibody index.

the respective order in the donors with MI and AI and 29 and 78 in donors with MoI and SI (Figures 3 and 4).

Similar results of the index value were obtained during subsequent plasma donations among donors with MI and AI and donors with MoI and SI, suggesting that there is no statistical significance between the value of the antibody index and the clinical severity of the disease.

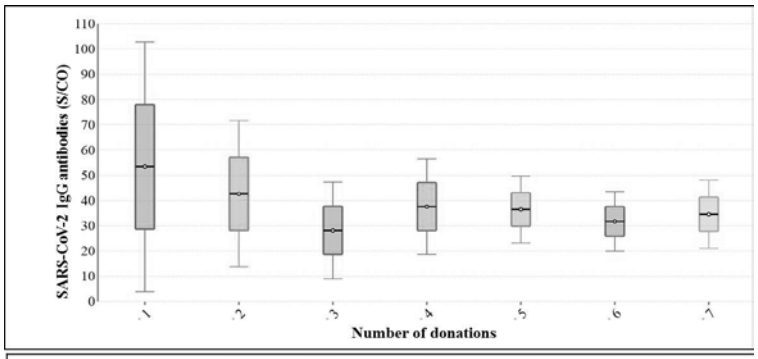
Among the 311 donors, there were 15 (4.8%) with AI, 235 (75. 6%) with MI, 45 (14.5%) with MoI, 16 (5.1%) with SI, and 0 with CI (Table 1).

The correlation of clinical severity and antibody titer analyzed by the Pearson correlation test showed a value of 0.2575, which does not confirm a statistically significant correlation of the variables.



SARS-CoV-2 IgG antibodies (S/CO) in donated plasma, obtained from people who have recovered from asymptomatic or mild COVID-19							
№ of donations	1	2	3	4	5	6	7
№ of donors	131	46	27	21	11	5	3
Min S/CO	1.02	10.61	8.61	6.46	6.71	9.33	9,84
Max S/CO	117.03	63.21	52.39	41.45	31.59	25.42	12.32

Fig. 3 – A range of anti-SARS-CoV-2 antibody (IgG) index among plasma donors who recovered from asymptomatic/mild illness.



SARS-CoV-2 IgG antibodies (S/CO) in donated plasma, obtained from people who have recovered from moderate or severe COVID-19							
№ of donations	1	2	3	4	5	6	7
№ of donors	33	12	7	5	4	3	3
Min S/CO	3.97	13.68	9.05	18.62	23.15	19.89	21.12
Max S/CO	102.82	71.63	47.29	56.58	49.76	43.56	48.04

Fig. 4 – A range of SARS-CoV-2 antibody (IgG) index among plasma donors who recovered from moderate/severe illness.

Table 1

Distribution of convalescent plasma donors according to clinical severity of COVID-19 and antibody index value

Antibody index	Clinical severity				Total n (%)
	Asymptomatic infection	Mild illness	Moderate illness	Severe illness	
1–6	6	29	3	0	38 (12.22)
7–26	6	100	10	5	121 (38.90)
27–46	2	63	15	5	85 (27.33)
47–66	1	35	11	4	51 (16.40)
67–86	0	5	3	2	10 (3.22)
87–106	0	2	3	0	5 (1.61)
≥ 107	0	1	0	0	1 (0.32)
Total n (%)	15 (4.82)	235 (75.56)	45 (14.47)	16 (5.15)	311 (100)

In all donors, the values of the antibody index decreased from the second to the last donation, but plasma donors with a more severe clinical manifestation of COVID-19 had stable antibody levels for a longer period of time.

Discussion

The study investigated the presence of SARS-CoV-2 IgG in 311 plasma donors. The study is based on the fact that a high titer of total IgG antibodies (anti-S and anti-N) implies an equally high titer of neutralizing antibodies that have a significant protective role in the immune response to viral infection and that IgG titer could also affect the clinical severity. The correlation between the number of antibodies detected after recovery and the clinical severity of the disease, as well as studies related to the duration of the high level of antibodies after the infection, are still the topic of scientific debates [10–13].

When taking note of anamnesis from plasma donors, special attention was paid to the symptoms they had during the disease. The highest percentage of our donors (75.6%) was found to have mild symptoms such as fever, cough, sore throat, headache, myalgia, and loss of sense of taste and smell, while only 4.8% of donors were asymptomatic. This representation of mild or asymptomatic donors is similar to the representation of donors in the convalescent plasma collection programs of other countries [14, 15]. In the donors of this group, the symptoms quickly withdrew, after which they reported no further complaints, while the laboratory findings were within normal limits. Donors with MoI (14.5%), of whom seven (2.25%) were hospitalized, had signs of pneumonia as well as accompanying symptoms on X-ray or CT scans. Donors from the SI group (5.1%) were the least represented in the convalescent plasma collection program, primarily because the severity of their illness required hospitalization with oxygen support and longer recovery time after discharge. No critically ill donor with applied invasive mechanical ventilation was present. A higher prevalence of donors under the age of 50 (83.9%) may also be associated with faster recovery from illness consequences and better psychophysical readiness to be included in plasma collection procedures.

The frequency of plasma donation during the study period depended on several factors, with the most significant value certainly being the SARS-CoV-2 IgG antibody index. However, the place of residence also significantly impacted the frequency of donations. The collection of convalescent plasma during the study was performed exclusively at the Institute for Blood Transfusion of Vojvodina as it was not possible to form an adequately equipped mobile unit that would collect plasma throughout the territory of Vojvodina. Transportation was organized for donors outside this district, but this fact was still a limiting factor for more frequent plasma donations. For this reason, the largest number of plasma donors came from the territory of the South Bačka district, where the Institute is located. In addition, 60.4% of donors residing outside the South Bačka district donated plasma only once.

Upon the first arrival, apart from other laboratory analyses, each donor underwent a rapid chromatographic test for antibodies, which, like most others, is based on lateral flow detection. The rapid test was positive in 60.1% of donors who donated plasma. Compared with the SARS-CoV-2 IgG antibody index, the limit of detection of the rapid test was 19.75, although in two donors with index values of 23.25 and 23.87, the rapid test was negative. Research shows that one-step delivery of the target analyte and detection reagents limit their accuracy [16]. In other infectious diseases detection, multi-step paper-based platforms, in which delivery of the target analyte was time- and volume-controlled, were used [17, 18]. That is considered to be the possible reason for the lower specificity and sensitivity of rapid anti-SARS-CoV-2 antibodies detection tests, which, according to previous research, detect only a high antibody titer [16].

To demonstrate the presence of SARS-CoV-2 IgG antibodies in plasma donors, a qualitative ELISA assay using SARS-CoV-2 recombinant antigens of solid-phase structural proteins (S and N) was used. Although the ELISA index value (ratio between sample and cut-off, S/CO) is expressed by a number, it does not show the level of antibodies, so the result is expressed as positive, negative, or indeterminate (± 10% of index value). In contrast, semi-quantitative and quantitative tests show the level of antibodies in the blood [titers, arbitrary units per milliliter (AU/mL), unit per milliliter (U/mL)]. However, it is important to note that the results of qualitative and quantitative assays are comparable as, in both cases, they depend on the analytical sensitivity of the test [19, 20].

Virus neutralization remains the gold standard for the determination of antibody efficacy. Szabó et al. [20] compared virus neutralization activity and results of anti-SARS-CoV-2 serological tests in plasma donors. Among the tests which showed the best sensitivity to neutralization was the test used in our study. They suggested the ELISA test as the first-pass test to rule out potential plasma donors with insufficient levels of neutralizing antibodies.

Dulipsingh et al. [21] used a quantitative assay to detect antibody titer and stated that > 6.5 AU/mL corresponds with an IgG antibody titer of about 1 : 320. In our study, selecting plasma donors based on antibody level was done for several reasons. First, during the early days of the pandemic (April-May 2020) in Serbia, the first and the only available test for screening plasma donors was the rapid test, and a little bit later, the test for detecting the presence of total antibodies against SARS-CoV-2. Early reports suggested that the total antibody test indicated a humoral response to COVID-19 infection. At that time, FDA guidelines did not require antibody testing, considering that the plasma collected from donors who recovered from the COVID-19 infection had quite enough neutralizing antibodies. Second, different studies observed that antibodies to both nucleocapsid and spike are correlated in the same patients [22, 23]. At the same time, no specific protocol for antibody testing was provided by FDA, and the minimum recommended titer was 1 : 80. In following recommendations, FDA determined 1 : 320 titer as the minimum level

required to achieve the therapeutic effect of plasma. Later, the requirement was reduced to 1 : 160 [5]. Our National protocol recommended the collection of COVID-19 convalescent plasma for therapeutic purposes if the least antibody index level was 6, according to manufacturer instructions. Although the BTIV collected plasma with such antibody levels, this plasma was not used in the treatment of patients. It should be emphasized that the testing was carried out at the very beginning of the epidemic when only qualitative tests were available. At that time, manufacturers were still developing other types of tests.

The humoral immune response is most important in eliminating cytopathogenic viruses and plays a major role in the prevention of viral reinfection [24]. Neutralizing IgG antibodies produced by B lymphocytes can be an indicator of protective immunity. Studies of the titer of neutralizing antibodies in infections with other coronaviruses show that over time the titer level slowly decreases: in the course of one year in influenza virus, three years in SARS-CoV, and two years after MERS-CoV [25]. Duration of antibodies after SARS-CoV-2 infection is still the subject of many studies, some of which show a decline in neutralizing antibodies within 2–3 months [26] and in IgG antibodies against the receptor-binding domain of spike protein within 75 days [27]. On the other hand, some studies question short-term immunity after infection and speculate that people with a more severe clinical manifestation had longer-lasting immunity than people with mild or asymptomatic clinical features [28]. Several Serbian authors describe a case report of a progressive decrease in IgG values for 6 months after COVID-19 infection [29]. In our study, no correlation was found between the clinical severity and the antibody titer in plasma donors. The analysis determined no statistically significant correlation between these two observed parameters. During the initial testing, the largest number of donors, 205/311 (65.9%), had a titer less than half the value of the highest titer (36.15), while the median was 26.07. The highest antibody index in our study was found in donors with a mild form of the disease, while 38.90% of donors with severe illness had lower values of antibody index. Studies show that the titer of neutralizing antibodies is not different between mild/moderate and severe cases [30–33] and that this is a significant deviation from SARS-CoV infections where antibody titer and clinical severity correlate [34–37].

A possible limitation of our study could be the sample size that was analyzed. A significant finding of the study is that during the observed time interval in all donors with severe and moderate clinical manifestation who repeatedly donated plasma, the antibody titer had slight oscillations. In mild or asymptomatic cases of donors, antibodies progressively decreased during the observed period. These results are consistent with data from the available literature [26, 33, 35]. Recovered individuals with a severe clinical manifestation developed a strong immune response by producing competent neutralizing antibodies [36]. Although there is no definite explanation, one of the possible reasons is that the specific anti-SARS-CoV-2 antibodies require enhanced and prolonged stimulation of B cell receptors, which occurs in patients with severe disease symptoms, and, thus, antibody titer values remain present for a longer period of time [38].

Conclusion

The study found that anti-SARS-CoV-2 antibodies were present in the sample of recovered patients, plasma donors, for more than 6 months after the disease. Even though no statistically significant correlation was found between the anti-SARS-CoV-2 antibody index value and the clinical severity of COVID-19, it has been proven that in patients with a more severe clinical manifestation, antibody values are maintained for a minimum of six months, which was the observed period. The data obtained are encouraging both for convalescent plasma collection programs and in terms of contribution to collective immunity. Characteristics of immunity developed following SARS-CoV-2 infection remain a topic for further research.

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Conflict of interest

The authors declare no conflict of interest.

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The frequency of cervical dentine hypersensitivity and possible etiological factors in an urban population: a cross-sectional study

Učestalost cervikalne dentinske preosetljivosti i potencijalnih etioloških faktora u gradskoj populaciji: studija preseka

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Abstract

Background/Aim. Dentine hypersensitivity (DH) is a commonly encountered clinical problem characterized by short, sharp pain which arises from exposed dentine. The aim of this study was to estimate the frequency of cervical DH in adults in Pančevo, Serbia, evaluate the correlation between DH and severity of cervical tooth wear, and investigate the impact of certain etiological factors. **Methods.** The study included 394 subjects, who were clinically examined and interviewed about potential etiological factors using a specially designed questionnaire. The presence of cervical DH was evaluated using cold air stimulation and Schiff ordinal scale. The severity of cervical tooth wear was evaluated using the Basic Erosive Wear Examination (BEWE). Results were analyzed using χ^2 tests and logistic regression at a significance level of $p \leq 0.05$. **Results.** The presence of cervical DH was recorded in 32.9% of the total number of subjects. The χ^2 analysis showed a significant association between clinically elicited and questionnaire-declared DH ($p < 0.001$), but not with the presence of noncarious cervical lesions and the extent of cervical tooth wear. Cervical DH showed a positive correlation with gender ($p < 0.001$), frequent consumption of citrus fruits ($p < 0.001$), and energy drinks ($p = 0.005$). Oral hygiene and other factors were not significantly associated. **Conclusion.** The prevalence of cervical DH in the investigated sample was relatively high. DH was more prevalent among females and significantly associated with frequent consumption of citrus fruits and energy drinks.

Key words:
dentin sensitivity; risk; serbia; surveys and questionnaire; tooth cervix.

Apstrakt

Uvod/Cilj. Dentinska preosetljivost (DP) je stanje koje se karakteriše kratkim i oštrim bolom usled izloženosti dentina. Cilj rada bio je da se utvrdi učestalost DP cervikalne regije zuba kod odraslih osoba iz Pančeva, Srbija i da se proceni uticaj stepena trošenja zubne supstance i drugih etioloških faktora na pojavu tog stanja. **Metode.** Studijom su obuhvaćena 394 ispitanika, koji su klinički pregledani i intervjuisani o potencijalnim etiološkim faktorima uz pomoć posebno kreiranog upitnika. Osetljivost cervikalne regije zuba ispitivana je primenom hladnog vazduha i procenjivana Šifovom numeričkom skalom. Procena stepena trošenja cervikalne zubne supstance vršena je primenom indeksa trošenja zuba (*Basic Erosive Wear Examination* – BEWE). Rezultati su analizirani primenom χ^2 testa i logističke regresije na nivou značajnosti $p \leq 0,05$. **Rezultati.** Prisustvo cervikalne DP dijagnostikovano je kod 32,9% ispitanika. Zabeležena je statistički značajna povezanost između klinički dijagnostikovane DP i osetljivosti zuba, prijavljene u anamnezi ($p < 0,001$), ali ne i sa prisustvom nekarijesnih oštećenja cervikalne regije zuba i stepenom trošenja zubne susptance. Pokazana je pozitivna korelacija DP sa polom ($p < 0,001$), učestalom konzumacijom citrusnog voća ($p < 0,001$) i energetskih napitaka ($p = 0,005$), a nije utvrđena korelacija sa ostalim faktorima, kao što je održavanje oralne higijene. **Zaključak.** U ispitanoj populaciji zabeležena je relativno visoka učestalost DP. Značajno viša učestalost zabeležena je među ispitanicima ženskog pola i onima koji često konzumiraju citrusno voće i energetske napitke.

Ključne reči:
dentin, osetljivost; rizik; srbija; ankete i upitnici; zub, vrat.

Introduction

The definition of a condition known as dentine hypersensitivity (DH), with minor adjustments, dates from 1983. It is characterized by short, sharp pain arising from exposed dentine in response to thermal, evaporative, tactile, osmotic, or chemical stimuli and cannot be ascribed to any other form of dental pathology. A suggestion from the Canadian Advi

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sory Board on Dentine Hypersensitivity was to change the term pathology into disease. Common terms used to describe this condition are also dentinal, cervical, cemental root sensitivity, or hypersensitivity [1].

Several theories, such as direct innervation theory, odontoblast receptor theory, or hydrodynamic theory, tried to explain the mechanism for DH, although neither leads to a complete understanding of how the various stimuli cause pain. The most widely accepted in the literature is a hydrodynamic theory, stating that the fluids within the exposed dentine tubules are disturbed by chemical or physical changes. Changes and movements of the intratubular fluid stimulate baroreceptors present in the pulp and dentin, which lead to neural discharge and result in painful sensations. For the development of DH, dentin and tubules must become exposed to the oral environment [2].

In most studies, gingival recession, chronic periodontal disease, frequent acidic dietary intake, and oral hygiene factors are considered risk factors for the occurrence of DH. Numerous authors agreed that the etiology is multifactorial and that interactions between several factors play an important role in initiating this condition [3]. A possible association between the presence of noncarious cervical lesions (NCCLs) and cervical DH was also evaluated in some studies. Both conditions are encountered frequently in dental practice and present a challenge for successful treatment. NCCLs and cervical DH occur in the same site of the tooth and, therefore, may be linked. It has been revealed that both conditions are supposed to be produced by a combination of erosion, abrasion, and attrition. However, there is still a lack of data, and differences in clinical characteristics and etiological factors on NCCLs and cervical DH need to be explored further [4].

The aim of this study was to estimate the frequency of cervical DH in an adult population sample in Pančevo, Serbia, to investigate the impact of possible etiological factors on the frequency of DH, and to evaluate the correlation between DH and severity of tooth cervical wear.

Methods

A cross-sectional study was conducted on a sample of patients selected by convenience sampling method, who approached the Department of Restorative Dentistry and Endodontics, Faculty of Dentistry in Pančevo, Serbia, for routine dental examination and possible treatment. The study involved adult patients aged over 18 years of both genders. The inclusion criteria for the participants were the following: a) to have a minimum of eight eligible teeth, b) absence of systematic diseases, and c) to be able to read and understand the questionnaire used in this study. Exclusion criteria were the following: dental bleaching procedures performed in the last 6 months, presence of large quantities of calculus on teeth, ongoing orthodontic treatment, and medication with sedatives, drugs, or desensitization agents that could affect the threshold of pain. The sample size was determined using the statistical power analysis program "G*Power 3.1" [5]. The calculation was based on the pilot study with a preliminary sample of 30 subjects, selected by convenience sampling method, as proposed by Browne [6], who approached routine dental examination and possible treatment. The calculation was done according to the sample analysis, and the proportion of the respondents with and without clinically elicited DH was 30% vs. 70% and 1.4 or higher odds ratio values for most of the tested variables. Alpha was set to 5%, and the power of 0.80 was considered acceptable. According to these parameters, a sample size of at least 344 participants would be required. The final study sample included 394 subjects (169 male and 225 female) divided into three age groups. The study was conducted in complete accordance with the World Medical Association's Declaration of Helsinki. Prior to the investigation, participants were fully informed about the study and gave written consent to participate as a volunteer. Investigations in this study were approved by the Ethics Commission of the Faculty of Dentistry in Pančevo (Approval Protocol No. 882/1–2014, according to Resolution sections 3, 7, and 8 of the National Commission of Ethics in Research).

Each participant completed a specially designed questionnaire created by researchers of this study and similar to those employed in previous studies, identifying etiological factors for DH and NCCLs [4, 7]. It included basic personal information and questions related to potential etiological factors, such as daily erosive dietary intake, carbonated and energy drinks consumption, bruxism and other bad habits, smoking, lifestyle, oral hygiene habits (daily tooth brushing frequency, bristle type, brushing movements, etc.). A test-retest correlation on a preliminary sample of subjects at two distinct periods was used to test the reliability of the questionnaire. The correlation coefficient (r) was 0.86, which was considered good.

Each patient was subjected to clinical examination for cervical DH and tooth wear. The presence of cervical DH was tested on all eligible teeth, excluding third molars, endodontically treated teeth, crowned teeth, and teeth with cervical caries and restorations. The cervical region of the tooth was subjected to cold air stimulation for 2 sec from a triple air dental syringe and a distance of approximately 1 cm. Adjacent teeth were shielded by the fingers of the other hand. The subject's response to the stimulus was evaluated using the Schiff ordinal scale (0 = subject does not respond to sensitivity, 1 = subject responds to stimulus but does not request discontinuation, 2 = subject responds to stimulus and requests discontinuation or moves from the stimulus, 3 = subject responds to stimulus, considers stimulus to be painful and requests discontinuation) [8]. The procedure was repeated for each eligible tooth. NCCLs were evaluated using the Basic Erosive Wear Examination (BEWE). Buccal/facial and lingual/palatal surfaces on all eligible teeth were examined, and the scores were given as follows: 0 = no surface loss, 1 = initial loss of enamel surface texture, 2 = distinct defect, surface loss < 50%, and 3 = surface loss > 50%. Only the highest score for each teeth sextant was recorded. After all the

sextants had been assessed, the cumulative score of all sextants was calculated [9].

The investigation was conducted by a single examiner, previously instructed regarding DH evaluation using the Schiff ordinal scale and NCCLs evaluation using the BEWE index. An intra-examiner agreement was calculated after examination of the preliminary sample, two times with an interval of two weeks, following recommendations from the World Health Organization (WHO) for reliability and validity of data. Cohen's Kappa value index was 0.92, which is considered excellent.

The collected data were analyzed using the statistical software SPSS v20.0 (IBM Inc, USA). Descriptive statistics for Schiff values were expressed as numbers and percentages for the respective groups (gender, age). Subject-level analysis was used to evaluate the association between possible etiological factors and clinically elicited DH. For that purpose, all subjects were divided into two groups regarding the presence of DH (maximum Schiff value 1-3) or absence (maximum Schiff value 0). The relationship between the presence of NCCLs, the severity of cervical tooth wear, questionnaire-declared hypersensitivity, and clinically elicited DH was estimated using the χ^2 test. The association of other possible etiological factors from the questionnaire with the clinically elicited DH was analyzed using logistic regression. Each factor was first employed as an independent variable in a univariate unconditional logistic regression, with the presence of DH as a dependent variable. Factors that showed significant correlation were then used as independent variables in the multivariate logistic analysis. The strength of association was presented by odds ratio (OR) at a significance level of $p \leq 0.05$ with a 95% confidence interval (CI). The logistic regression model was reviewed for goodness-of-fit and validated using the Hosmer-Lemeshow statistics.

Results

The study included 394 patients – 169 males and 225 females. The youngest one was 19, the oldest one was 81, and the average age of the study sample was 45.4. The presence of cervical DH (maximum Schiff value ≥ 1) was diagnosed in 32.9% of subjects, while the frequency of NCCLs was 68.5%. The presence of both cervical DH and NCCLs was diagnosed in 22.8% of the total number of subjects. Among 69.2% of subjects with cervical DH, the presence of NCCLs was also registered (Table 1).

Table 1

Frequency of subjects with cervical dentine hypersensitivity (DH) and noncarious cervical lesions (NCCLs)

Parameter	DH (maximum Schiff value ≥ 1)		Total n (%)
	yes n (%)	no n (%)	
NCCLs			
yes	90 (22.8)	180 (45.7)	270 (68.5)
no	40 (10.2)	84 (21.3)	124 (31.5)
Total	130 (32.9)	264 (67.1)	394 (100)

DH was more frequently (43.6%) diagnosed among females than among males (18.9%). In contrast, a higher percentage of subjects with at least one NCCL was recorded among males (76.3%) than among females (62.7%) (Figure 1). The frequency of subjects with cervical DH was almost equally distributed among age groups, with the highest frequency in the group of 36–54 years (38.8%). The percentage of subjects with NCCLs increased with age, with a frequency of 94.7% in the group over 55 years of age (Figure 2).

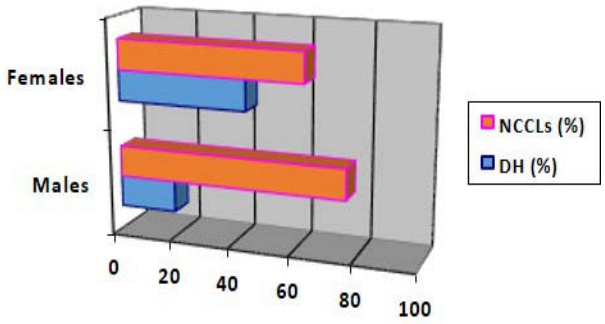

Fig. 1 – Distribution of subjects with cervical dentine hypersensitivity (DH) and noncarious cervical lesions (NCCLs) concerning gender.

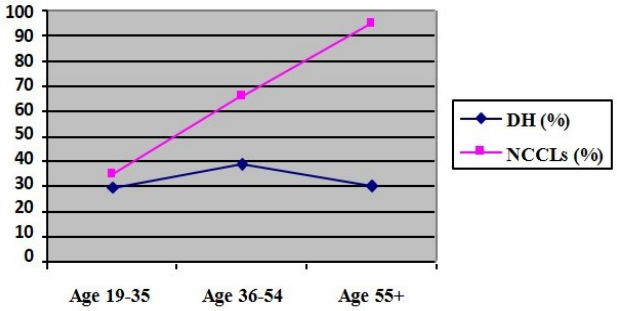

Fig. 2 – Distribution of subjects with cervical dentine hypersensitivity (DH) and noncarious cervical lesions (NCCLs) concerning age.

The majority of male subjects (81.1%) and more than half of females (56.4%) did not respond to cold air stimulation (Schiff value = 0). Between those who responded in some manner, the less intensive response to a stimulus (Schiff value = 1) was the most frequent among both genders and all three age groups. The most intensive response (Schiff value = 3) was recorded only among females (3.6%) and subjects over 55 years of age (5.3%) (Table 2).

Table 2

Response to cold air stimulation concerning gender and age

Parameter	Maximum Schiff value, n (%)			
	0	1	2	3
Gender				
male	137 (81.1)	24 (14.2)	8 (4.7)	0 (0)
female	127 (56.4)	64 (28.4)	26 (11.6)	8 (3.6)
Age (years)				
19–35	74 (70.5)	21 (20.0)	10 (9.5)	0 (0)
36–54	85 (61.2)	42 (30.2)	12 (8.6)	0 (0)
55+	105 (70.0)	25 (16.7)	12 (8.0)	8 (5.3)
Total	264 (67.1)	88 (22.3)	34 (8.6)	8 (2.0)

Table 3

Association between clinically elicited cervical dentine hypersensitivity (DH) and presence of noncarious cervical lesions (NCCLs), cervical tooth wear, and questionnaire-declared hypersensitivity

Paramater	Total number	Cervical DH n (%)	Odds ratio	95% CI	χ^2	*p*
Presence of NCCLs						
no	124	40 (32.3)	1			
yes	270	90 (33.3)	1.05	0.67–1.65	0.044	0.833
Maximum BEWE score						
0	124	41 (33.1)	1			
1	132	36 (27.3)	0.759	0.44–1.29	3.194	0.313
2	89	33 (37.1)	1.193	0.67–2.11	0.760	0.544
3	49	21 (42.9)	1.518	0.77–2.99	2.328	0.228
Declared DH						
no	297	55 (18.5)	1			
yes	97	76 (78.3)	15.924	9.05–28.02	117.941	< 0.001

CI – confidence interval; BEWE – Basic Erosive Wear Examination.

Table 4

Results of univariate and multivariate logistic regression for the presence of cervical dentine hypersensitivity (DH)

Factors	Elicited DH	Univariate logistic regression analysis			Multivariate logistic analysis		
	n (%)	OR	95% CI	*p*	OR	95% CI	*p*
Gender							
male	33 (19.5)	1			1		
female	98 (43.5)	3.180	2.00–5.05	< 0.001	2.774	1.71–4.49	< 0.001
Age (years)							
19–35	31 (29.5)	0.977	0.57–1.69	0.935			
36–54	54 (38.8)	1.528	0.94–2.49	0.088			
55+	45 (30.0)	1					
Citrus fruits							
no/rarely	22 (16.7)	1			1		
often	109 (41.6)	3.562	2.12–5.99	< 0.001	3.285	1.87–5.76	< 0.001
Fruit juices							
no/rarely	72 (35.6)	1					
often	59 (30.7)	0.801	0.53–1.22	0.301			
Carbonated drinks							
no/rarely	96 (35.0)	1					
often	35 (29.2)	0.763	0.48–1.22	0.256			
Energy drinks							
no/rarely	116 (31.8)	1			1		
often	15 (51.7)	2.300	1.07–4.92	0.032	3.657	1.47–9.11	0.005
Milk							
no/rarely	22 (39.3)	1					
often	109 (32.2)	0.736	0.41–1.32	0.302			
Chewing gums							
no/rarely	54 (36.2)	1					
often	77 (31.4)	0.806	0.53–1.24	0.326			
Smoking							
no	86 (38.2)	1			1		
yes	45 (26.6)	0.587	0.38–0.90	0.016	0.715	0.44–1.15	0.168
Parafunctions							
no	96 (33.6)	1					
yes	35 (32.4)	0.949	0.59–1.52	0.828			
Brushing frequency							
1 per day	26 (26.0)	0.586	0.25–1.36	0.214			
2 per day	52 (37.7)	1.008	0.45–2.23	0.985			
≥ 3 per day	12 (37.5)	1					
Toothbrush							
soft	20 (31.2)	0.559	0.23–1.38	0.207			
medium	47 (36.4)	0.705	0.31–1.59	0.401			
hard	13 (44.8)	1					
Movements							
vertical	31 (36.9)	1					
horizontal	22 (37.9)	1.045	0.52–2.08	0.901			
circular	20 (37.0)	1.006	0.49–2.04	0.987			
variable	58 (29.3)	0.708	0.41–1.21	0.209			
Brushing after meal							
no	92 (30.1)	1			1		
yes	39 (44.3)	1.851	1.14–3.01	0.013	1.454	0.85–2.48	0.167

OR – odds ratio; CI – confidence interval.

The χ^2 analysis showed a significant association between clinically elicited sensitivity (maximum Schiff value ≥ 1) and questionnaire-declared hypersensitivity ($p < 0.001$). A significant association between the presence of cervical DH and the presence of NCCLs was not recorded ($p = 0.833$). The presence of cervical DH showed a growing tendency with the severity of cervical tooth wear, but the association was not statistically significant (Table 3).

The results of univariate unconditional logistic regression showed a direct link between the presence of cervical DH and gender ($p < 0.001$), frequent consumption of citrus fruits ($p < 0.001$) and energy drinks ($p = 0.032$), smoking cigarettes ($p = 0.016$), and brushing teeth immediately after a meal ($p = 0.013$). After conducting the multivariate logistic regression analysis, only gender ($p < 0.001$) and frequent consumption of citrus fruits ($p < 0.001$) and energy drinks ($p = 0.005$) were associated with the presence of cervical DH (Table 4).

Discussion

There is a growing awareness in the literature that DH represents an important issue that affects the quality of life of many individuals worldwide. Equally important is to study this condition both from a diagnostic and therapeutic perspective. The majority of researchers used cold air stimulation from a triple-air dental syringe or scratching the tooth with a dental probe to provoke DH-associated pain. The patient's response to the presenting stimulus has been the primary way for evaluation because of the ability to quantify the condition using a pain rating scale [10–12].

There is a wide range of data in the literature regarding the prevalence of DH. Rahiotis et al. [13] tried to explain that by a certain factor related to the design issues of each study, such as different types of stimuli applied to provoke the sensitivity, the methodology for assessing DH, the number and the profile of the participants, and different settings (general practice or university/hospital clinic). Recent review paper reported a prevalence range from 1.3% to 92.1%. Summary estimation was 11.5% and 33.5% for the fixed and random-effects meta-analysis models, respectively [14]. The results of the present study revealed a prevalence of 32.9%, which corresponds to that estimation.

A high discrepancy in the literature is also present regarding the prevalence of NCCLs, with a range between 9.1% and 93%. In the systematic review, Teixeira et al. [15] estimated a mean prevalence of 40.7%, with a higher prevalence (54%) in studies with older populations. The prevalence of NCCLs in the present study (68.5%) was higher than average but similar to the results of two studies from close geographical regions, which revealed a prevalence of 65% and 52% [16, 17].

Despite the assumption of similar etiology, epidemiological studies that correlate the presence of NCCLs and cervical DH are not very common due to the difficulty in comparing data from different populations. Several studies revealed a significant association between the presence of cervical DH and NCCLs [4, 13, 18, 19]. Cunha-Cruz et al. [20] have not found a significant association between the two conditions, which was also the case in the present study. The reason could be the fact that NCCLs were not considered separately by type (erosion, abrasion, abfraction) but cumulatively. The association between the severity of tooth wear and cervical DH is still scarce in the literature. Some studies found a positive correlation [21–23]. Reasons could be the proximity of the lesion with the pulp, the amount of exposed dentinal tubules, and the theory that root exposition makes the tissue more vulnerable to the influence of risk factors [24]. In the present study, despite a positive correlation between the presence of cervical DH and the severity of cervical tooth wear, the association was not statistically significant. A possible explanation could be the fact that not all exposed dentine is sensitive. There must be an opening of the dentinal tubule system to permit activation of the hydrodynamic mechanism by appropriate stimuli [23]. NCCLs development tends to be a slow, chronic process that occurs over an extended period. Therefore, sclerosis, lack of secondary dentin deposition, occlusion of open dentinal tubules, pulpal retreat, and other natural tooth-protective measures slowly adapt to the noxious stimuli and minimize sensitivity [25].

Clinically elicited cervical DH and questionnaire-declared hypersensitivity were significantly associated in the present study. Self-reported prevalence (24.6%) was lower compared to clinically elicited DH (32.9%). A similar result was obtained in the West et al. [22] and Barroso et al. [26] studies, which tried to explain the fact that the severity of pain is proportional to the stimulus strength, the subject's psychological state, and anxiety of the expected pain. Moreover, most of the clinically tested subjects showed less intensive response to a stimulus (maximum Schiff value = 1), suggesting no interference of pain in daily life. In contrast, Savage et al. [23] reported a higher prevalence of questionnaire-declared DH compared to clinically diagnosed.

The results of the present study indicated a significantly higher prevalence of cervical DH among females. However, the reasons are not yet clear, but it could be because females have better overall healthcare and oral hygiene awareness. That could make them become more sensitive, evoke different responses to stimuli or lower pain threshold, anticipate pain differently, and show a tendency to eat acidic food more frequently [4, 13, 24, 27]. It should be mentioned that there are studies in which significant difference between genders was not recorded [2, 22, 23]. It is fair to say that the correlation between age and the prevalence of cervical DH is still unclear. Although the reported peak in the literature is between 20 and 50 years, many researchers concluded that there is a tendency for this to decline with age, mainly because of the age-related changes in the dentin-pulp complex. In addition, the fact that the extent of periodontal diseases and gingival recession that cause cervical DH increases with age must be considered; that could lead to higher prevalence among older patients [2, 13]. The highest frequency of cervical DH in the present study was recorded among middle-aged subjects (36–54 years) but without significant association.

The influence of acidic dietary intake on the occurrence of NCCLs and cervical DH is often evaluated in epidemio-

logical studies. The cervical tooth region is prone to erosion because of the proximity to the gingival margin and weak ability to self-cleaning. Therefore, the erosive potential of acidic dietary products lasts for a longer period. High concentrations of processed carbohydrates, stimulation of higher levels of acid production, and higher titratable acidity are the reasons why soft drinks, fruit juices, and energy drinks have erosive potential. As a result of dentine erosion, the smear layer disappears, and dentinal tubules become opened [1]. In the present study, frequent consumption of citrus fruits and energy drinks was significantly associated with the presence of cervical DH. Similar results were obtained by West et al. [22], Savage et al. [23], and López et al. [28]. Yoshizaki et al.[18] found a significant correlation between frequent consumption of acidic fruit juices and DH diagnosed with air blast, while there are a certain number of studies in which significant association with acidic dietary intake was not recorded [13, 15, 29].

Cervical DH can occur among patients with good oral hygiene and limited bleeding on probing. Gingival recession can result from aggressive and inappropriate tooth brushing, which exposes dentine to further abrasive wear and increases the risk of developing DH. Many researchers tried to establish the connection between tooth brushing habits and the prevalence of DH, but it seems that a clear association could not be established, probably due to the condition's multifactorial nature. Scaramucci et al. [30] found that subjects who brush their teeth four times a day and those who apply excessive force during brushing could be more prone to DH but could not find a correlation with the hardness of different brush bristles. Among all evaluated oral hygiene factors, Que et al. [4] found only frequent tooth brushing as a risk factor for DH. In the present study, besides a slightly higher prevalence of cervical DH among subjects who use hard toothbrushes, horizontal movements, and brush their teeth immediately after a meal, a significant association was not recorded. Similar results were obtained in a number of other studies [13, 20, 22, 24, 29].

The effect of smoking on the development of periodontal destruction is well established. However, the data from the present study found no such association, which was also the case in studies by Rahiotis et al. [13] and Mahdisiar et al. [31]. In some studies, parafunctional habits, such as bruxism, were also reported as contributing factors to the occurrence of cervical DH. A possible explanation could be that during parafunctional loading, cyclic tension and compression stresses occur in the cervical tooth region, which leads to the loss of tooth structure at the cemento-enamel junction and consequently the occurrence of hypersensitivity [32]. Furthermore, the assumption is that bruxers exhibit an altered perception of painful stimuli as a result of increased levels of anxiety and depression [2, 30 33]. No significant association between cervical DH and parafunctional habits was recorded in the present study. Teixeira et al. [24] also found no significant association.

Some limitations of the present study should be considered. Many investigators have suggested using at least two methods in the diagnosis of cervical DH, such as tactile and air-blast stimulation, due to the strong placebo effect and subjectivity of pain response. In the present study, tactile stimulation was not used since it is difficult to standardize the applied pressure, which could lead to the fact that, even with a negative response to a tactile stimulus in the clinical environment, patients may still have DH-pain caused by mechanical stimuli in their everyday life [11]. Although the study sample consisted of subjects from the region, it could not be considered nationally representative. However, the results from cross-sectional studies are useful for estimating the frequency of cervical DH and identifying etiological factors in certain regions, which could be used as a starting point for further longitudinal evaluations involving a larger sample, nationally representative.

Conclusion

Within the limitations of this study, it can be concluded that the prevalence of cervical DH in the investigated sample was relatively high. The factors associated with the presence of cervical DH were female gender and frequent consumption of citrus fruits and energy drinks. Clinically elicited cervical DH was significantly associated with the questionnaire-declared hypersensitivity but not with the presence of NCCLs and the severity of cervical tooth wear. Obtained results support findings on the multifactorial nature of cervical DH.

Conflict of interest

The authors declare no conflict of interest.

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The cuspal deflection caused by dental composite polymerization shrinkage analyzed by digital holography

Ispitivanje uticaja polimerizacione kontrakcije dentalnog kompozita na deformaciju kvržica zuba digitalnom holografijom

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Abstract

Background/Aim. Polymerization shrinkage of filling materials is one of the main disadvantages of adhesive restorative dentistry. The objective of the study was to measure tooth cusps deflection caused by polymerization shrinkage of a resin-based dental material (RDM) in real-time using digital holographic interferometry (DHI) in two groups of cavities restored with and without an additional wall. Simultaneously, internal tooth mechanical behavior was monitored. **Methods.** Standardized three class I cavities were prepared on third molar teeth. The teeth were cut in two halves in the longitudinal plane, obtaining six samples for the study (now with class II cavities), divided into two groups (group G1 – with the additional wall, group G2 – without it), and mounted in aluminum blocks. The cavities were filled with the RDM, cured with a light emitting diode (LED) for 40 sec from the occlusal direction, and monitored during the curing and post-curing period using DHI. Data were analyzed using the Student's *t*-test for independent samples and the Anderson-Darling test, with an alpha level of 0.05. **Results.** At the end of the examined period, the samples from the group G1 showed significantly increased tooth cusps deflection [t (10) = 4.7; p = 0.001] compared to samples from the group G2. **Conclusion.** Within the limitations of this study, it was concluded that the presence of the additional wall simulating a dental matrix band had a significant influence on the increase and prolonged deflection of tooth cusps during the examined RDM polymerization shrinkage.

Key words:
composite resins; interferometry; polymerization; tooth.

Apstrakt

Uvod/Cilj. Polimerizaciona kontrakcija dentalnog materijala za ispunu je jedan od glavnih problema u restorativnoj stomatologiji. Cilj rada bio je da se detektuje naprezanje kvržica zuba izazvano polimerizacionom kontrakcijom dentalnog materijala na bazi smole (DMS) u realnom vremenu, primenom digitalne holografske interferometrije (DHI) u dve grupe kaviteta restauriranih sa i bez dodatnog zida. Uporedo je praćeno mehaničko ponašanje zubnog tkiva. **Metode.** Na humanim trećim molarima pripremljena su tri standardizovana kaviteta klase I. Zubi su presečeni na dve polovine u uzdužnoj ravni. Dobijeno je šest uzoraka (sada sa kavitetima klase II) podeljenih u dve grupe (grupa G1 – sa dodatnim zidom, grupa G2 – bez njega) i fiksiranih u aluminijumske kalupe. Kaviteti su potom ispunjeni DMS, osvetljeni tokom 40 s svetlosno-emitujućom diodom (*light emitting diode* – LED) iz okluzalne projekcije i ispitivani tokom i nakon perioda osvetljavanja primenom DHI. Podaci su analizirani Studentovim *t*-testom za nezavisne uzorke i Anderson-Darlingovim testom, sa nivoom značajnosti alfa od 0,05. **Rezultati.** Na kraju ispitivanog perioda, uzorci iz grupe G1 pokazali su značajno veću deformaciju kvržica zuba [t (10) = 4,7; p = 0,001], u poređenju sa uzorcima iz grupe G2. **Zaključak.** U okviru ograničenja ove studije, zaključeno je da je prisustvo dodatnog zida, simulirajući dentalnu matricu, značajno uticalo na povećano i produženo naprezanje kvržica zuba prilikom polimerizacione kontrakcije ispitivanog DMS.

Ključne reči:
smole, kompozitne; interferometrija; polimerizacija; zub.

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Introduction

Despite the continuous improvements in the field of resin-based dental materials (RDM), polymerization shrinkage of filling materials is one of the main disadvantages that remains of interest in adhesive restorative dentistry. During material setting, polymerization shrinkage stress (PSS) is generated at the tooth-restoration interface, which is considered responsible for several negative clinical effects that may arise, including debonding, leakage, postoperative sensitivity, secondary caries, cusps deflection, and crack formation in enamel/dentin [1, 2]. Regarded as a physical process, PSS depends on several factors such as volume/weight percentages of the resin matrix, filler formulation, restorative procedure, and cavity configuration (C-factor) [3]. Hence, PSS is a highly non-uniform and multifactorial phenomenon that cannot be measured directly [4–6]. Consequently, experimental tests need to be carried out to investigate the constraints imposed on the bonded restorations [7–10] and to estimate interface problems in adhesive reconstructions caused by PSS [10, 11]. On the other hand, direct monitoring of secondary phenomena, such as tooth cusps deflection, can provide indirect vision into PSS development [12–16].

Digital holographic interferometry (DHI) is a laser optic technique suitable for non-destructive and contactless measurements of submicron changes in highly asymmetrical objects with micrometer precision [16, 17]. The efficiency of classical holographic interferometry in the field of dental biomechanics was previously studied [18, 19], while DHI is a relatively new testing method. Due to the digital nature of the method (digital camera, computer software), enabling fast and simple recording and reconstruction of holographically generated interference images, DHI has become a valuable tool in different fields of science and technology [20].

The bulk of the mineralized tooth tissue is composed of dentin which supports the overlying hard and brittle enamel in the part of the tooth crown [21]. These two specific calcified tissues are joined by the dentin-enamel junction (DEJ), described as a natural multilevel interface that plays a vital role in the accommodation of stress [21–23]. Considering the anisotropic histological structure of enamel and dentin, it is of utmost importance for the clinical practice to appreciate the impact of PSS on each of the surrounding hard tooth tissues.

On the other hand, it was previously identified that confinement imposed on the RDM by bonding to tooth cavity walls affects the level of PSS [8, 9]. This specific relationship, described through the concept of the C-factor and defined as the ratio of bonded to unbonded (free) surfaces of the restoration [24], still contributes to layering restorative procedures [25, 26] or bulk filling techniques [27]. Meanwhile, during proximal tooth cavity reconstruction, the creation of the missing tooth part is built by using a metal band (matrix band) to perform a proper tooth crown reconstruction. Therefore, the impact of this additional constraint on the RDM, caused by the matrix band, was examined in this study.

The objective of this study was to measure tooth cusps deflection during the RDM polymerization shrinkage, in real-time using DHI, in two groups of cavities restored with and without an additional wall. Simultaneously, internal tooth mechanical behavior throughout the curing and post-curing period of the RDM was monitored. Following the aforementioned aim, the hypothesis that there is no significant difference in tooth cusps deflection at the end of the examined period between the two groups was presumed.

Methods

Ten third molar teeth extracted for pericoronitis, periodontal disease, or orthodontic reasons were collected before the beginning of the experiment at the Department of Oral Surgery, Dentistry Clinic of Vojvodina, Novi Sad, Serbia. The teeth were cleaned of residual periodontal ligament and debris and stored in distilled water (23 ± 1˚C). All clinical procedures were performed under the ethical guidelines of the Ethics Committee of the Faculty of Medicine, University Novi Sad (No 01-39/32/1, from April 15, 2021). Due to numerous anatomical variances of third molar teeth, all gathered teeth were measured following the previously defined criteria by Politi et al. [28] – a maximum buccal-palatal width (BPW) of 10.25–10.75 mm, and the presence of four cusps (two buccal and two palatal). As a result, three third molar teeth were included in the study.

Sample preparation

Class I cavities were prepared on the three selected teeth using a high-speed handpiece (300,000 rpm) with water spray and a round diamond bur for cavity preparation with perpendicular walls to the pulp floor and rounded internal line angles. In the interest of better control of the biological variability of human teeth, cavity preparation was performed following relative rather than absolute measures as follows: the width was two-thirds of the BPW, the occlusal isthmus was prepared to half of the BPW, the mesial-distal extension was performed towards the end of the central groove preserving marginal ridge integrity, and the axial depth was set at 2 mm (measured from the end of the central groove). In that manner, the integrity of the tooth cusps and marginal ridges was preserved, avoiding potential inconsistency in tooth tissue mechanical behavior during PSS.

To estimate the mechanical behavior of internal tooth tissue, teeth were cut in half to expose dentin, enamel, and DEJ since DHI can only visualize surface changes of non-transparent objects for the wavelength of the selected light source [16]. Samples were cut longitudinally (vestibule-oral direction) in two halves according to the study of Xia et al.[16] resulting in six samples for the analysis with two tooth cusps each (buccal and palatal), permitting the internal tooth mechanical behavior evaluation. In the next step, the samples were mounted in aluminum blocks using dental gypsum (Marmorock 20; Dr. Böhme & Schöps™ GmbH, EN 26873/ ISO 6873, type IV) to the level of the enamel-cement junction, ensuring the visibility of the tooth crown and appropriate mechanical stability necessary for the experiment. Before applying the resin bonding adhesive system (Single bond universal adhesive® – 3M™ Deutschland GmbH, LOT No.

663414), the cavities were cleansed under copious water irrigation and etched with phosphoric acid (Gel etchant 37.5% phosphoric acid, Kerr Italia™ s.r.l, LOT No. 3596305) using the total-etch technique (30 sec enamel, 15 sec dentin) and then water-rinsed once again. The bonding system was then applied following the manufacturer's instructions and cured for 10 sec with a LED light source (SmartLite IQ2® LED curing light, Dentsply®, Model No. 200, Serial No. B21581, 500 mW/cm^2).

Two groups of three samples each were formed so that one-half of every tooth was included in the first group (G1) and the second group (G2), respectively. On the G1 group samples, a piece of a thin microscopic cover glass was added along the proximal side of the cavity during tooth fixation, simulating a matrix band (normally used in clinical practice to restore class II cavities) and maintaining the visibility of internal tooth tissues, while the samples in the G2 group were mounted without it serving as a negative control. The prepared cavities were restored by the bulk filling technique, using a single increment of material per cavity.

Resin-based dental material

For the RDM, a nano-filler resin composite was used (Filtek Ultimate Universal Restorative®), A2 body shade, 3M ESPE ™ USA, lot No. N867954, matrix: bisphenol-A-diglycidyl ether dimethacrylate (bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), bisphenol-A-polyethylene glycol diether dimethacrylate (bis-EMA), polyethylene glycol dimethacrylate (PEGDMA). The filler consisted of non-agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated 4–11 nm zirconia filler, and aggregated zirconia/silica cluster filler (average cluster particle size 0.6–10 μm). Filler loading was 78.5% by weight and 63.3% by volume.

Finally, samples were fixed in the DHI setup. After fixation, the polymerization process was activated with the LED light source, applied from the occlusal direction at a distance of 1mm from the sample surface, and using a continuous curing mode of 40 sec. The study included the examination of the curing and post-curing period lasting a total of 320 sec (~ 5 min).

Experimental setup

The tooth cusps deflection was directly monitored in real-time using a custom-made DHI setup with a single laser beam expanded by a diverging lens and a spherical mirror (Figure 1). In that manner, observation and laser light illumination of the sample from both sides (front and rear) was enabled, while the region of interest was the cut side of the sample, providing a vision of the internal tooth tissues (Figures 2a–3b). By generating all the necessary beams from the same input beam, excellent mechanical stability of the setup required for the holographic experiment was obtained [17]. In this experiment, a diode-pumped solid-state, frequency-doubled Nd: Vanadate (Nd: YVO_4) laser was used that provided single-frequency green output (Coherent® Verdi V5, 532 nm wavelength, 5 W maximum power). The power output of 500 mW was enough for this experiment, while the linewidth of the laser was less than 5 MHz, guaran-

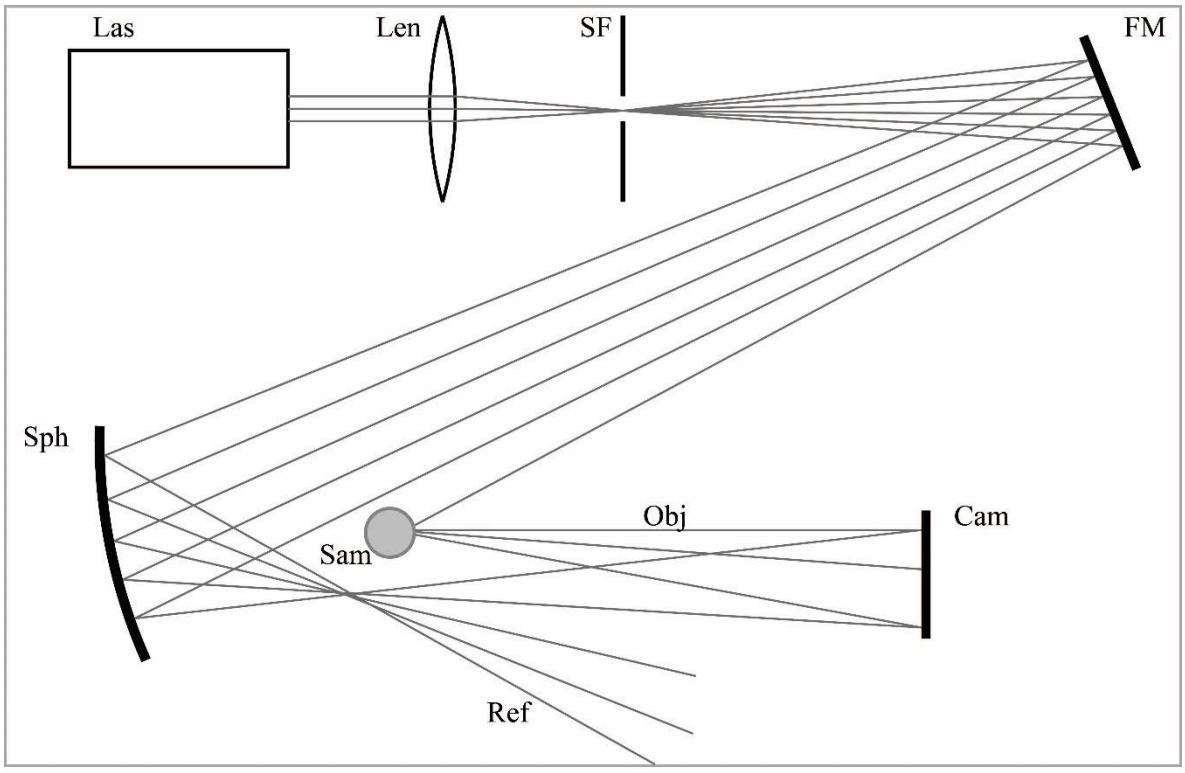

Fig. 1 – Custom made digital holographic interferometry (DHI) setup.
Las – laser; Len – lens; SF – spatial filter; FM – flat mirror; Sph – spherical mirror; Sam – sample; Ref – reference beam; Obj – object beam; Cam – camera.

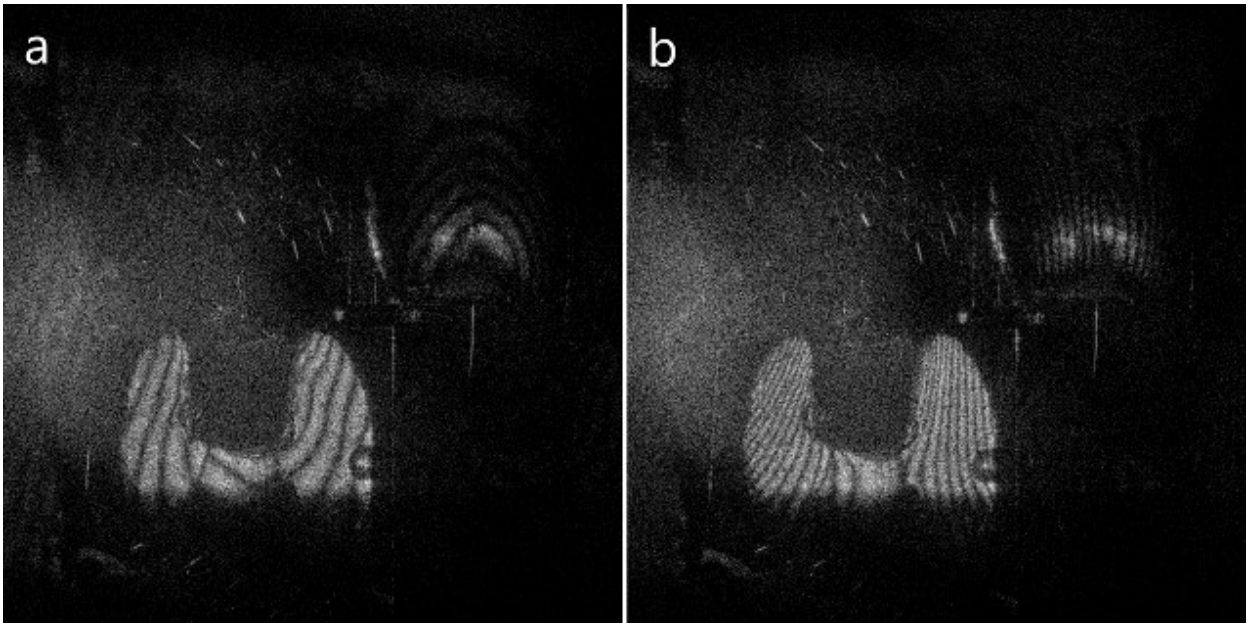

Fig. 2 – Interference pattern in group G1: a) at the end of the curing period (42 sec); b) at the end of the whole examined period (320 sec).

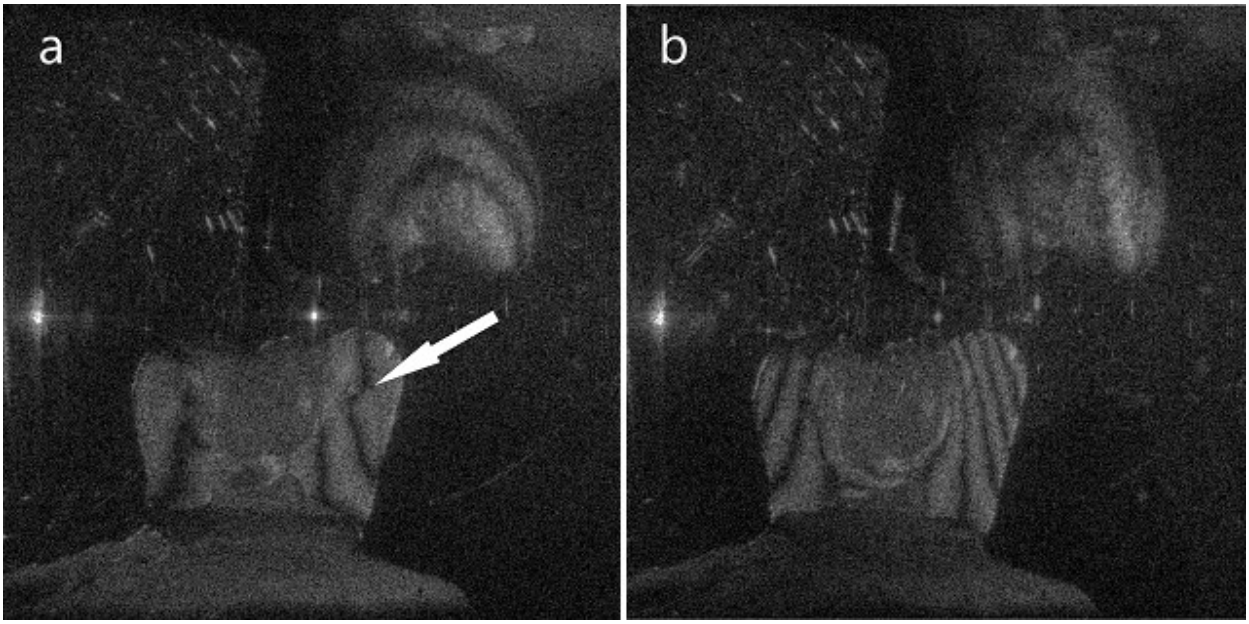

Fig. 3 – Interference pattern in group G2: a) at the end of the curing period (42 sec) – white arrow indicating the change in fringe appearance; b) at the end of the whole examined period (320 sec).

teeing a highly coherent beam. A digital single-lens reflex camera (Canon® EOS50d, 15.1 megapixels, 4752 × 3168 image size) recorded the resulting holograms (every 2 sec during the first minute and 10 sec afterward until the end of the observation period). The obtained images were transferred to a computer and numerically reconstructed by parallel processing on a graphic card (NVidia® GeForce GTX 1060 6GB) [17].

The holographic experiment was based on the interference between the object beam (scattered from the object) and the reference beam (one that misses both the front and rear side of the object and continues to propagate) generated from the same radiation source. Due to the existing movement of the sample (tooth cusps deflection), the reconstructed holographic interferograms showed a specific interference pattern presented in the form of a series of interference lines (so-called "fringes") whose number, shape, and orientation gave information about the resulting mechanical deformation. The exact amount of deformation was calculated by multiplying the number of fringes that appeared in the examined interval of time with the wavelength of laser light [17].

Statistical analysis

Statistical analysis was performed in Minitab® software (version 19.2020.1; 64-bit) using the Student's *t*-test for in-

dependent samples (n = 6 + n = 6, G1 and G2, respectively) for testing the presumed hypothesis. The distribution normality of the results was analyzed by the Anderson-Darling tests. Power calculations were carried out for the Student's *t*-test. An alpha level of 0.05 was used for all statistical tests.

Results

The resulting DHI images (interferograms) from both groups presented a specific interference pattern indicating tooth cusps deflection, with each fringe appearing corresponding to deformation of 0.532 μm (Figures 2a–3b). At the end of the examined period, the G1 group samples restored with the cover glass [n = 6; Mean (M) = 5.4; Standard deviation (SD) = 1.6] showed a significantly higher amount of cusps deflection per cusp [t (10) = 4,7; p = 0.001] than the samples from the G2 group restored without it (n = 6; M = 2.1; SD = 0.6).

Table 1 summarizes the measured single values of cusps deflection. The cusps deflection reached a maximum of 7.8 μm and 2.7 μm per cusp in the groups G1 and G2, respectively (Figure 4). The Anderson-Darling test showed that data from both the G1 (AD = 0.2; p = 0.6) and G2 groups (AD = 0.3; p = 0.4) followed a normal distribution. Power calculations, carried out for the Student's *t*-test, showed that the chosen sample size allowed registering differences between groups at < 3 μm (2.9 μm) with the power of 0.87 (87%).

The results also provided qualitative information about the submicron movements of the examined samples – during the curing reaction of the RDM in some samples from the group G2, a change in fringe appearance was noticed when moving along the DEJ projection (Figure 3a).

Discussion

The results of this study contributed to the investigation of the biomechanics of tooth cusps displacement in class II adhesive restorations during PSS development. Utilizing DHI, direct measurement of submicron tooth cusps dis-

Table 1

Measured single values for each tooth cusp, at the end of the whole examined period (final tooth cusps deflection)

	Sample number	Tooth cusp	Final cusps deflection (μm)	Standard deviation (SD)	Sample size (n)
First group (G1)	1	Buccal	4.79	1.6	6
		Palatal	3.72		
	2	Buccal	5.85		
		Palatal	3.72		
	3	Buccal	7.80		
		Palatal	6.39		
Second group (G2)	4	Buccal	2.66	0.62	6
		Palatal	1.06		
	5	Buccal	1.6		
		Palatal	2.13		
	6	Buccal	2.13		
		Palatal	2.66		

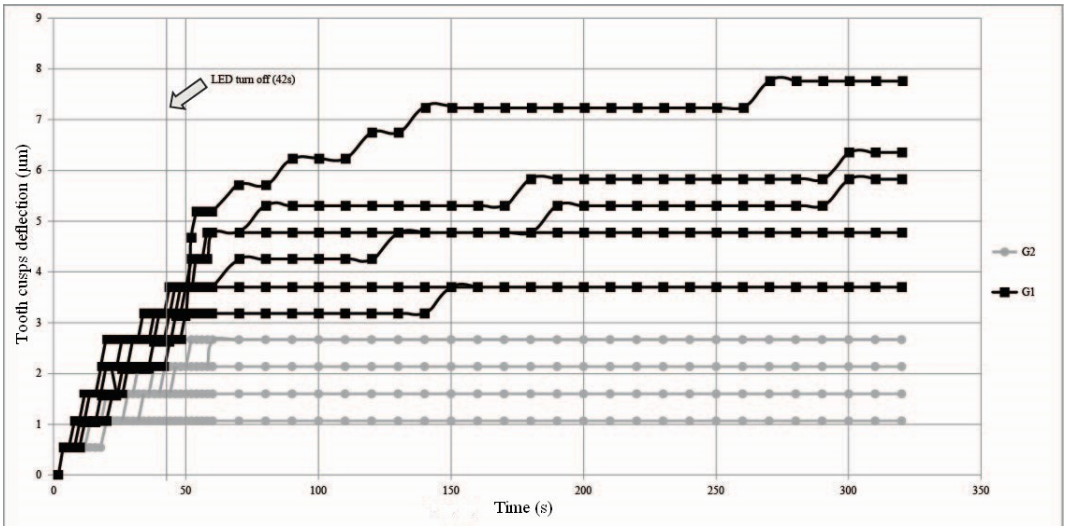

Fig. 4 – Tooth cusps deflection (μm) during the examined period as a function of time. G1 – group 1 samples restored with the additional wall; G2 – group 2 samples restored without the additional wall; LED – light emitting diode.

placement was performed, enabling indirect monitoring of the polymerization reaction kinetics. When the interferograms of the two groups were compared at the same moment of recording (end of the curing period and the whole examined period), it was observed that the interferograms presented more fringes in the G1 group than in the G2 group (Figures 2a–3b). This result indicated increased tooth cusps displacement in the G1 group (on average 5.4 μm per cusp versus 2.1 μm in G2). Assuming the same experimental conditions in the two groups, such as standardized cavities, RDM, restorative technique, and polymerization protocol, the occurrence of increased cusps deflection in the G1 group was associated with the cavity configuration (C-factor) variation due to the presence of an additional glass wall.

The results also showed that cusps deflection did not finish at the end of the curing period but continued to increase in the post-curing period (Figure 4). That indicated that the polymerization reaction continued after the photoactivation step, as already demonstrated by several studies [29–31]. When the post-curing deformation per cusp in the groups G1 and G2 was compared, it was evident that in the group G1, it continued to increase gradually until the end of the examined period, unlike the absence of such progress in the group G2 (Figure 4). Nevertheless, the research conducted by Germscheid et al. [31] stated that examination of the post-curing period of contemporary RDMs should cover a time interval longer than 1 h (up to 15 hrs) due to the significant amount of measured post-curing shrinkage. Further evaluation in a prolonged period could be a topic of another study, using a modified examination protocol by recording the interference images every 10–15 min, thereby rationalizing hard-disk memory storage.

However, the marginal adaption of RDMs in class I and II cavities reflects complex interactions between adhesive bonding on the one hand [25] and PSS at the tooth-restoration interface on the other [32]. The level of PSS and debonding are more probably dependent upon the shape and hence constraints of the cavity [8, 9, 33, 34], as well as viscoelastic properties of RDMs [35, 36] than other factors. It is well known that cavity configuration (C-factor) is one of the main factors affecting the development of PSS [8, 9, 33, 37] since greater confinement imposed on the RDM leaves a smaller number of free surfaces for resin composite shrinkage and PSS relaxation. According to the study of Han et al. [8], the RDMs can shrink relatively freely in a cavity with a larger number of unbonded surfaces. These findings have been shown using different tools in several studies where post-gel PSS was evaluated [9, 38–40], not only tooth cusps displacement. Apart from the effect of the C-factor and RDM elastic modulus upon PSS, a relevant role was also found in adhesive filling techniques [13, 28, 41]. In the present study, a bulk-filling technique was used to avoid sample movement during the examination and preserve the mechanical stability of the setup necessary for the holographic experiment.

Even though the samples in the G1 group were not bonded to the cover glass, this additional wall transformed their cavity configuration, which increased and extended cusps deflection (following PSS), imitating class I cavity configuration [42, 43]. Control of glass stability was performed manually for every sample in the G1 group before filling the cavity. Based on the presented results, the first null hypothesis, stating that there is no significant difference in tooth cusps deflection at the end of the examined period between the two proposed groups (with and without an additional wall), was rejected.

On the other hand, a qualitative assessment of the resulting interference images revealed the change in fringe appearance at the DEJ projection in some samples from the group G2 during the curing reaction of the RDM (Figure 3a). This finding could highlight the role of DEJ in the accommodation of internal forces such as PSS, as suggested by the results of several studies [16, 22, 23]. However, the presence of a regular interference pattern and the influence of the additional wall, in this sense, are yet to be explored.

Given the aggravating circumstances of conducting a clinical study that would establish a direct link between the phenomenon of PSS with certain clinical outcomes, in vitro studies play a significant role in the field of RDM examination due to the need for constant improvement of materials on the market. Therefore, this study proved that DHI, as a non-destructive method with submicron precision, is able to directly investigate the tooth cusps deformation and predict PSS influence on the behavior of adhesively restored molar teeth.

Conclusion

Based on the obtained results and within the limitations of this study, it was concluded that, by changing cavity configuration, the presence of an additional glass cover wall simulating a dental matrix band, had an influence on increased and prolonged tooth cusps deflection during the polymerization reaction of the RDM. Future perspectives would be to explore if any regular pattern in the behavior of tooth tissue under internal stress, such as PSS, could be found, especially in the presence of an additional matrix band wall.

Acknowledgment

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Importance of four-dimensional computed tomography simulation in locally advanced lung cancer radiotherapy: impact on reducing planning target volume

Značaj simulacije četvorodimenzionalnom kompjuterizovanom tomografijom u radioterapiji lokalno uznapredovalog karcinoma pluća: uticaj na smanjenje planiranog ciljnog volumena

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Abstract

Background/Aim. Four-dimensional (4D) computed tomography (CT) simulation is a useful tool for motion assessment in lung cancer radiotherapy. Conventional three-dimensional (3D) free-breathing (FB) simulation is static, with limited motion information on respiratory movements that can produce inaccuracies in the delineation process and radiotherapy planning. The aim of this study was to compare clinically significant differences between the target volumes defined on 3D CT vs. 4D CT simulation and the potential impact on the planning target volume (PTV), bearing in mind that a reduced PTV with precise coverage of the primary tumor is extremely important. In addition, quantification of movements of the primary tumor (gross tumor volume – GTV) was performed during 4D CT simulation on three axes: Z-superoinferior (SI), X-mediolateral (ML), and Y-anteroposterior (AP). **Methods.** This retrospective study evaluated 20 lung cancer patients who underwent CT simulation for radical radiotherapy treatment. FB 3D CT and 4D CT simulations were acquired for each patient in accordance with our institutional protocol. A volumetric comparison of radiation volumes defined on 3D CT vs. 4D CT simulation was done on the following: GTV 3D vs. internal GTV (IGTV) 4D and PTV 3D vs. internal PTV (IPTV) 4D. The comparison of GTV movement in the FB phase GTV (GTV FB), phase 0 (GTV 0), phase 50 (GTV 50), and phase maximum intensity projection (GTV MIP) was made with GTV FB as the basic value. The evaluation was made on all three axes. **Results.** The comparison of volumetric values between GTV 3D vs. IGTV 4D was 63.15 cm^3 vs. 85.51 cm^3 ($p < 0.001$), respectively. IGTV 4D was significantly larger than GTV 3D ($p < 0.001$). The mean value of equivalent spherical diameter (ESD) for PTV 3D vs. IPTV 4D was 8.44 cm vs. 7.82 cm ($p < 0.001$), respectively, and the mean value volume PTV 3D vs. IPTV 4D was 352.70 cm^3 vs. 272.78 cm^3 ($p < 0.001$), respectively. PTV 3D was significantly larger than IPTV 4D ($p < 0.001$). A statistically significant difference ($p < 0.05$) was identified in the deviation related to the Z-axis between the upper and lower lobe. **Conclusion.** 4D CT simulation-based delineation can reduce PTV compared to 3D simulation-based radiation therapy; therefore, it is a prerequisite for high-quality and precise radiation therapy treatment.

Key words:
adenocarcinoma; carcinoma, squamous cell; four-dimensional computed tomography; lung neoplasms; radiotherapy.

Apstrakt

Uvod/Cilj. Simulacija putem četvorodimenzionalne kompjuterizovane tomografije (4D KT) je važan segment savremene radioterapije karcinoma pluća. Konvencionalna trodimenzionalna (3D) simulacija uz slobodno disanje (*free-breathing* – FB) je statična sa limitiranim informacijama o respiratornim pokretima koji mogu produkovati nepreciznosti u procesu delineacije i planiranju radioterapije. Cilj ove studije bio je da se uradi poređenje ciljnih volumena definisanih na 3D KT simulaciji vs. 4D KT simulaciji i uticaja na planirani ciljni volumen (PCV), imajući u vidu da je

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smanjeni PCV uz preciznu pokrivenost primarnog tumora od izuzetne važnosti. Urađena je kvantifikacija pokreta primarnog tumora (*gross tumor volume* – GTV) tokom 4D KT simulacije duž tri ose: Z-superoinferiornu (SI), X-mediolateralnu (ML), Y-anteroposteriornu (AP). **Metode.** U ovoj retrospektivnoj studiji evaluirano je 20 pacijenata sa dijagnozom lokalno uznapredovalog karcinoma pluća i indikacijom za radikalnu radioterapiju. Prema institucionalnom protokolu uradjena je 3D KT i 4D KT simulacija FB za svakog pacijenta. Zatim je urađeno volumetrijsko poređenje volumena definisanih putem 3D KT vs. 4D KT: GTV 3D vs. unutrašnji GTV (UGTV) 4D i PCV 3D vs. unutrašnji PCV (UPCV) 4D. Poređenje pomeranja GTV u fazi FB (GTV FB), fazi 0 (GTV 0), fazi 50 (GTV 50) i fazi projekcije maksimalnog intenziteta, *maximum intensity projection* (GTV MIP) urađeno je tako da je GTV FB uzet kao bazična vrednost. Evaluacija je urađena za sve tri ose. **Rezultati.** Izmerene vrednosti volumena GTV 3D vs. UGTV 4D bile su 63,15 cm^3 vs. 85,51 cm^3 ($p < 0,001$). UGTV 4D je bio značajno veći u odnosu na GTV 3D ($p < 0,001$). Srednja vrednost ekvivalentnog sfernog dijametra (ESD) za PCV 3D vs. UPCV 4D bila je 8,44 cm vs. 7,82 cm ($p < 0,001$), srednja vrednost volumena PCV 3D vs. UPCV 4D bila je 352,70 cm^3 vs. 272,78 cm^3 ($p < 0,001$). PCV 3D je bio značajno veći u poređenju UPCV 4D ($p < 0,001$). Utvrđena je i statistički značajna razlika ($p < 0,05$) u odstupanju GTV u odnosu na Z osovinu između gornjeg i donjeg lobusa. **Zaključak.** Delineacija bazirana na 4D KT simulaciji daje mogućnost redukcije PCV u poređenju sa 3D simulacijom i čini važan preduslov za visoko kvalitetan i precizan radioterapijski tretman.

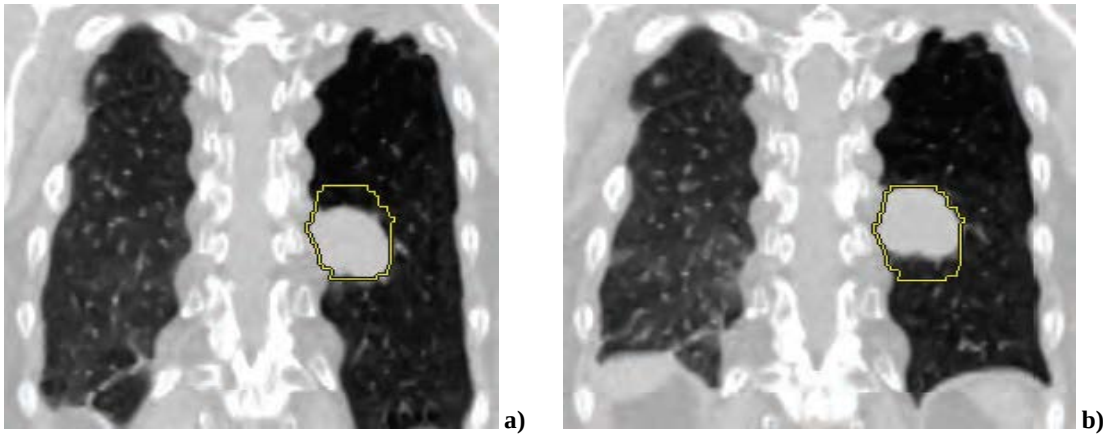
Ključne reči:
adenokarcinom; karcinom, planocelularni; tomografija, kompjuterizovana, četvorodimenzionalna; pluća, neoplazme; radioterapija.

Introduction

Lung cancer is the main cause of cancer death in men and women, presenting almost 20% of all cancer deaths [1]. Non-small cell lung cancer (NSCLC) is the most common histological form found in 85% of newly diagnosed lung cancer cases. In the first presentation, more than 60% of patients are shown in the stage of locally advanced or metastatic disease [2].

Locally advanced stage shows patients with clinical tumor, node, metastasis (TNM) status IIIA, IIIB, IIIC, and the real challenge for radiation therapy is to deliver radical dose to advanced and complex target volumes [3]. Radiotherapy has a crucial role in the radical treatment of locally advanced lung cancer. Its primary purpose is to administer higher doses to the tumor and lower to the surrounding organs at risk in order to maximize tumor control and minimize treatment-related toxicities [4]. In the 1980s, with the introduction of computed tomography (CT) into the planning process, 3D CT became the standard in lung cancer radiotherapy [5]. Assessment and accounting of respiratory motion are vital issues in lung cancer radiotherapy. Historically, respiratory motion management was resolved by adding large planning margins, which imposes a reduction of prescription doses below radical. Technological progress over the last decades with different motion compensation strategies provides more precise and accurate management of respiratory motion during radiotherapy treatment. Respiratory gating can be achieved with external markers (e.g., Varian RPM respiratory gating system) or internal markers as surrogates of tumor motion [6].

Intensity-modulated radiation therapy (IMRT) is a modern and innovative technology increasingly used in contemporary radiation therapy centers [7]. 4D CT simulation is an integral part of IMRT. Modern 4D CT scanners can image the whole thorax and capture all the respiratory cycle phases in less than a minute [8]. Scans obtained during the 4D CT simulation show that more than 50% of lung tumors move more than 5 mm during treatment, while 11% have movements larger than 1 cm [9]. The position of the tumor lesion during inspiration and expiration is shown in Figure 1. The 4D CT simulation enables superior visualization of the target volume and adequate monitoring of respiratory movements during the treatment planning and treatment delivery phase. There are two primary components of the motion: volumetric changes in the target and positional changes of the targets as well as the surrounding organs. The ultimate goal of varied motion management



a) **b)**

Fig. 1 – Tumor volume position during: a) inspiration; b) expiration.

strategies is to detect anatomical motion and adjust radiation therapy accordingly [10].

Due to variations in the position of the target volume during the respiratory cycle, it is impossible to account for the change of the target motion in the course of 3D free-breathing (FB) simulation. In this sense, there is a need to correct this geometric uncertainty in 3D CT. Consequently, these actions pose a larger definitive planning target volume and higher toxicity of surrounding organs at risk [11]. The primary aim of this study was to make a volumetric comparison of the target volumes defined in 3D CT vs. 4D CT simulation and the impact on definitive planning target volume (PTV) in locally advanced lung cancer radiotherapy. Additionally, the movement of gross tumor volume (GTV) in different phases with GTV FB as the basic value was observed in order to determine the significance of the movement assessment and 4D simulation for an adequate target volume coverage.

Methods

Patient selection

The study group included 20 patients with locally advanced lung cancer treated with radical radiotherapy intent at the Radiotherapy Department from September 2018 to January 2020. The patients were identified retrospectively, and 3D and 4D CT scan data were analyzed. The study was approved by the Institutional Review Board (No 03/2020, from October 06, 2020). All patients provided written informed consent prior to the treatment planning.

Data acquisition

3D CT and 4D CT images (2.5 mm slice thickness) were sequentially acquired for each patient. The simulation session was performed in an adequate treatment position (supine position with arms above the head) and with adequate immobilization (All-in-One breast- and lungboard solution – Orfit industries, Wijengem, Belgium) on a GE Lightspeed multislice CT (General Electric Medical System, Waukesha WI). The first CT images were acquired while the patient was FB in a 3D CT data set. 4D CT images were acquired after that using an external respiratory gating system (Real Time Positioning Management System-Varian Medical Systems Inc. Palo Alto, CA, USA). With Advantage 4D system application, sets of images were acquired and classified in ten equally divided breathing phases according to the respiratory cycle (0–100%) labeled as CT_0, CT_{10}, CT_{20}, … and CT_{90}.

Using the appropriate software tools on a 4D CT workstation, the synthesis of CT images was made and created: Max IP – maximum intensity projection, presenting any position where the tumor is present during all respiratory phases; Min IP – minimum intensity projection, presenting one position where the tumor is constantly present in all respiratory phases; Mean IP – mean intensity projection which represents the temporal presence of the tumor in certain respiratory phases.

Then, CT images were transmitted to Varian Eclipse (Varian Medical Systems, Palo Alto, CA, USA) treatment planning system (TPS).

3D CT-based delineation

Target volumes delineation was performed on the 3D CT FB data set according to our institutional protocol, International Commission on Radiation Units and Measurements ICRU 50 and 61 recommendations for 3D CT [12]. GTV 3D was manually contoured and encompassed the primary tumor lesion visualized on FB CT data set images at the window level. Clinical target volume (CTV) 3D was defined with 6–8 mm [squamous cell carcinoma (SCCA), 6 mm; adenocarcinoma (AC), 8 mm] expansion of GTV, including all subclinical lesions and possible areas of infiltration. Planning target volume (PTV) 3D was derived from CTV 3D plus conventional margin 1cm axial, 1.5 cm superior and inferior margin to incorporate setup errors and respiratory motion (Figure 2). The 3D radiotherapy plan is shown in Figure 3.

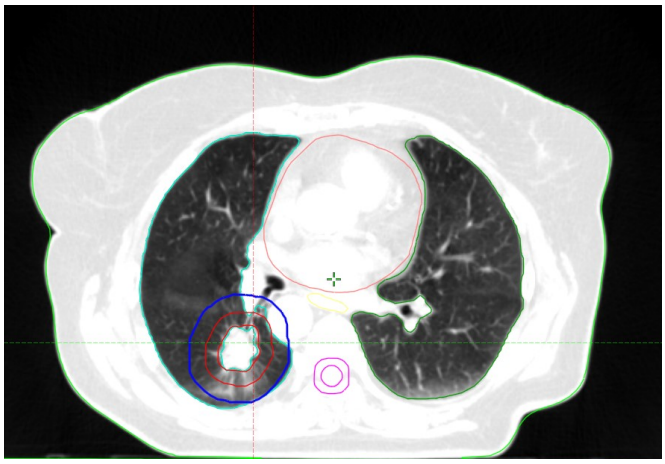


Fig. 2 – 3D conformal-based contouring.
Light red – gross tumor volume (GTV); dark red – clinical target volume (CTV); blue – planning target volume (PTV).

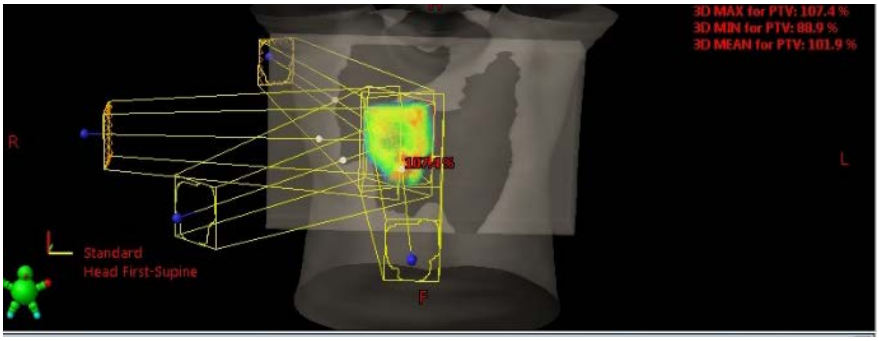


Fig. 3 – 3D conformal plan for radiotherapy treatment.

4D CT simulation-based delineation

4D CT-based radiotherapy volume definition was made following our institutional protocol and ICRU 83 recommendations for IMRT [13].

GTV 4D was manually contoured at the lung window level in different phases in 4D CT images: phase 0 – GTV 0 (which is the end of inspiration), phase GTV 50 (which is the end of expiration), GTV FB (free-breathing phase), GTV MIP (summation of average tumor position in all ten phases of the breathing cycles). For each patient, GTV 0, GTV 50, GTV FB, and GTV MIP were combined in one unique volume defined as internal – IGTV 4D, which represents the precise contour of GTV (Figure 4). Internal CTV (ICTV) 4D was defined with expansion IGTV 4D plus 6–8 mm (SCCA 6 mm, AC 8 mm) based on previous studies [14]. Internal PTV (IPTV) 4D was derived from ICTV 4D plus 0.5 mm isotropic expansion to incorporate setup error only. Delineation of surrounding organs at risk was performed (heart, oesophagus, spinal cord, lungs) according to RTOG recommendations and our institutional protocol [15]. IMRT plan is shown in Figure 5.

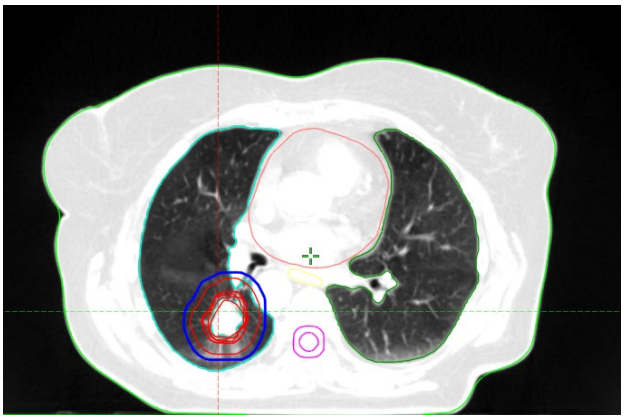


Fig. 4 – 4D intensity modulated radiation therapy (IMRT) contouring. Dark red – internal gross tumor volume (IGTV): GTV 0+GTV 50+GTV free-breathing (FB)+GTV maximum intensity projection (MIP); light red – internal clinical target volume (ICTV); blue – internal planning target volume (IPTV).

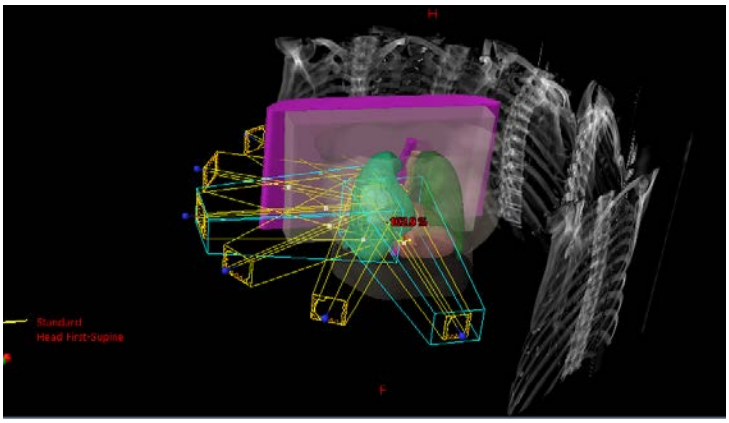


Fig. 5 – 4D-based intensity modulated radiation therapy (IMRT) plan for radiotherapy treatment.

Evaluation of 3D CT vs. 4D CT delineation data sets

Quantitative differences from both imaging data sets – 3D CT-based vs. 4D CT-based simulation were evaluated. Volumetric values GTV 3D, CTV 3D, and PTV 3D vs. volumetric values IGTV 4D, ICTV 4D, and IPTV 4D on two data sets expressed in cm^3 were evaluated. Equivalent spherical diameter (ESD) of GTV 3D, CTV 3D, and PTV 3D vs. ESD of IGTV 4D, ICTV 4D, and IPTV 4D on two data sets expressed in cm were evaluated. An evaluation was conducted by comparing both values.

Due to an objective possibility that a lower percentage of lungs would be irradiated in patients with larger lung volume and a higher percent of lungs would be irradiated in patients with a smaller lung volume when their PTV, CTV, and GTV are equal, the ratios of GTV/total lung, CTV/total lung, PTV/total lung were assessed and analyzed.

Total lung volume (TLV) is the bilateral lung volume (right lung volume plus left lung volume) and the volume of bilateral lungs excluding GTV 3D, CTV 3D, PTV 3D–GTV 3D/total lung ratio, CTV 3D/total lung ratio, and PTV 3D/total lung ratio was expressed in percentages.

TLV excluding IGTV 4D, ICTV 4D, IPTV 4D – IGTV 4D/total lung ratio, ICTV 4D/total lung ratio, and IPTV 4D/total lung ratio were expressed in percentages. Finally, evaluation was made by comparing the extracted volumes from 3D and 4D data sets.

Additionally, in this study, quantification of movements for primary tumor (GTV) during 4D IMRT in three axes was performed. A comparison of GTV movements was made with GTV FB as the basic value.

Statistical analysis

Measured data are presented in graphs and tables with original measurements or standard descriptive statistical measures – the sample means and sample standard deviations (mean ± SD). The data were analyzed by fitting general linear mixed models (GLMM), while in the case of significant differences in variation between the studied groups, an appropriate post-hoc analysis was conducted by testing variances between the means applying the standard pairwise test procedure. Pearson correlation coefficient (r) was also calculated. The results of the analysis were discussed in view of practical and statistical significance. Results of the analysis in terms of the observed differences between the studied groups were deemed statistically significant in case $p < 0.05$. Statistical analysis and graphical representation of data were prepared with the support of SPSS 22 software (IBM, 2013).

Results

The patients' characteristics are described in Table 1. A total of 20 patients were included and analyzed in this study. The average age of the study group was 70.8 years, with a range of 50–78 years. Most of the patients (60%) had the TNM stage IIIA. Eleven patients had pathological verified AC, and nine had SCCA. Fourteen patients were male, and six were female. Localization of the tumor was peripheral in 11 patients, and central localization was verified in 9 patients.

Statistical analysis of mean volumes generated on 3D and 4D data sets is shown in Table 2. The mean value of

Table 1

Patient characteristics

Parameters	Values
Total number of patients, n (%)	20 (100)
Age (years), mean (range)	70.8 (50–78)
Tumor, n (%)	
AC	11 (55)
SCCA	9 (45)
Gender, n (%)	
male	14 (70)
female	6 (30)
Tumor localization, n (%)	
central	9 (45)
peripheral	11 (55)
Lobe, n (%)	
RUL	4 (20)
RML	4 (20)
RLL	4 (20)
LUL	3 (15)
LLL	3 (15)
Lingula	2 (10)
TNM, n (%)	
III A	12 (60)
III B	5 (25)
III C	3 (15)

AC – adenocarcinoma; SCCA – squamous cell carcinoma; RUL – right upper lobe; RML – right middle lobe; RLL – right lower lobe; LUL – left upper lobe; LLL – left lower lobe; TNM – tumor, node, metastasis.

Table 2

A volumetric comparison of radiation volumes 3D simulation-based data set vs. 4D computed tomography-based simulation data set

Radiation volume	ESD (cm)		Volume (cm^3)		Lung/volume ratio (%)	
	Mean	SE	Mean	SE	Mean	SE
GTV 3D	4.40	0.34	63.15	14.43	1.63	0.42
IGTV 4D	5.12	0.31	85.51	16.96	2.20	0.50
CTV 3D	6.16	0.38	152.27	27.63	3.84	0.79
ICTV 4D	6.79	0.33	183.30	29.59	4.62	0.85
PTV 3D	8.44	0.38	352.70	46.65	8.78	1.34
IPTV 4D	7.82	0.32	272.78	36.39	6.81	1.05
F	364.76		43.33		32.65	
p	< 0.001		< 0.001		< 0.001	

GTV – gross tumor volume; IGTV – internal GTV; CTV – clinical target volume; ICTV – internal CTV; PTV – planning target volume; IPTV – internal PTV; ESD – equivalent spherical diameter; SE – standard error.

ESD GTV 3D vs. IGTV 4D was 4.40 cm vs. 5.12 cm ($p < 0.001$), respectively. The mean volumetric value of GTV 3D vs. IGTV 4D was 63.15 vs. 85.51cm^3 ($p < 0.001$), respectively. IGTV 4D was significantly larger than GTV 3D ($p < 0.001$).

The mean value of ESD CTV 3D vs. ICTV 4D was 6.16 cm vs. 6.79 cm ($p < 0.001$), respectively. The mean volumetric value of CTV 3D vs. ICTV 4D was 152.27 cm^3 vs. 183.30 cm^3 ($p < 0.001$), respectively. ICTV 4D was significantly larger than CTV 3D ($p < 0.001$) (Figures 6 and 7).

The mean value of ESD PTV 3D vs. IPTV 4D ESD was 8.44 cm vs. 7.82 cm ($p < 0.001$), respectively. The mean volumetric value PTV 3D vs. IPTV 4D ESD was 352.70 cm^3 vs. 272.78 cm^3 ($p < 0.001$), respectively. PTV 3D was significantly larger than IPTV 4D ($p < 0.001$) (Figures 6 and 7).

The mean value GTV 3D/total lung ratio vs. IGTV 4D/total lung ratio was 1.63 vs. 2.20 ($p < 0.001$), respectively. The mean value CTV 3D/total lung ratio vs. ICTV 4D/total lung ratio was 3.84 vs. 6.62 ($p < 0.001$), respectively.

The mean value PTV 3D/total lung ratio vs. IPTV 4D total lung ratio was 8.78 vs. 6.81 ($p < 0.001$), respectively. The results of these comparisons are shown in Table 2.

IGTV 4D/total lung ratio and ICTV 4D/total lung ratio were significantly larger than GTV 3D/total lung ratio and CTV 3D/ total lung ratio ($p < 0.001$), respectively.

The definitive PTV 3D/total lung ratio was significantly larger than IPTV 4D/total lung ratio ($p < 0.001$), respectively (Figure 8). Correlations between the measured variables are shown in Table 3.

Figure 9 shows a graphical presentation of the tumor position for each patient in the X, Y, and Z-axis. There was almost no dislocation along the X and Y-axes when assessed in phases GTV 0, GTV 50, GTV FB, or GTV MIP. Analysis of dislocation along the Z-axis indicated that the difference between the studied phases was highly significant ($p < 0.001$).

When different breathing phases were compared, a statistically significant difference in the movement along the X-axis ($p = 0.931$) was not identified. The result of the movement along the Y-axis was similar, with no statistically significant difference ($p = 0.524$).

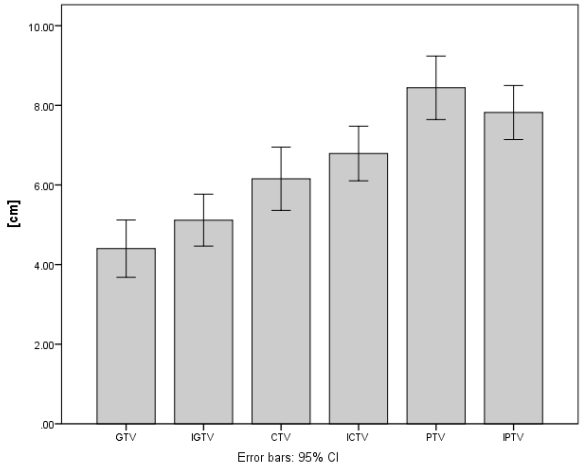


Fig. 6 – Comparison of equivalent spherical diameter (ESD) volumetric values of radiation volumes GTV 3D, CTV 3D, and PTV 3D vs. IGTV 4D, ICTV 4D, and IPTV 4D expressed in cm.
GTV – gross tumor volume; IGTV – internal GTV; CTV – clinical target volume; ICTV– internal CTV; PTV – planning target volume; IPTV – internal PTV; CI – confidence interval.

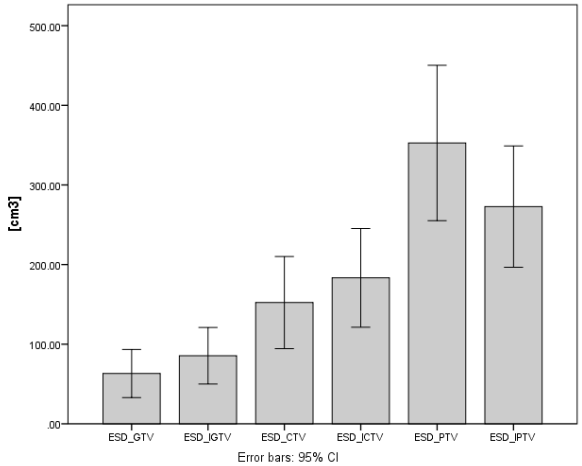


Fig. 7 – Comparison of values of radiation volumes for GTV 3D, CTV 3D, and PTV 3D vs. IGTV 4D, ICTV 4D, and IPTV 4D expressed in cm^3.
ESD – equivalent spherical diameter; GTV – gross tumor volume; IGTV – internal GTV; CTV – clinical target volume; ICTV – internal CTV; PTV – planning target volume; IPTV – internal PTV; CI – confidence interval.

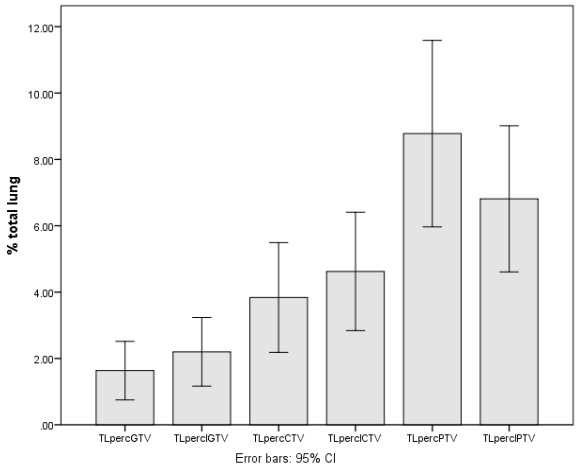


Fig. 8 – Comparison of GTV 3D, CTV 3D, and PTV 3D/total lung ratio vs. IGTV 4D, ICTV 4D, and IPTV 4D/total lung ratio expressed in percentages (%); CI – confidence interval. TL – total lung; GTV – gross tumor volume; IGTV – internal GTV; CTV – clinical target volume; ICTV – internal CTV; PTV – planning target volume; IPTV – internal PTV; CI – confidence interval.

Table 3

Correlation coefficients (r) between measured variables

Variables	GTV	IGTV	CTV	ICTV	PTV	IPTV
GTV	1	0.991	0.968	0.969	0.897	0.92
IGTV	0.991	1	0.967	0.974	0.91	0.93
CTV	0.968	0.967	1	0.988	0.973	0.977
ICTV	0.969	0.974	0.988	1	0.96	0.979
PTV	0.897	0.91	0.973	0.96	1	0.989
IPTV	0.92	0.93	0.977	0.979	0.989	1

GTV – gross tumor volume; IGTV – internal GTV; CTV – clinical target volume; ICTV – internal CTV; PTV – planning target volume; IPTV – internal PTV.

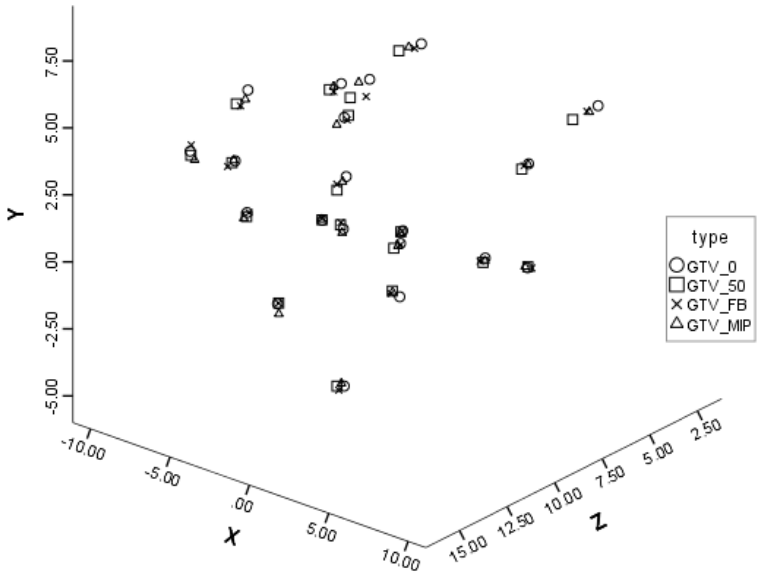


Fig. 9 – Graphical presentation of tumor position (GTV) in different breathing phases (GTV 0, GTV 50, GTV FB, GTV MIP) along X, Y, Z-axis during the breathing cycle; Z – superoinferior (SI), X – mediolateral (ML), Y –anteroposterior (AP). GTV– gross tumor volume; FB – free-breathing; MIP – maximum intensity projection.

The highest values were obtained in the GTV MIP phase, compared to all other phases. Phases GTV FB and GTV 50 did not differ significantly and were placed between GTV 0 and GTV MIP values.

Discussion

Precise and safe delivery of radiation dose to a tumor that moves with respiration is a real clinical and technical challenge. Technically, safe delivery of thoracic radiotherapy involves multiple factors: precise CT imaging, positioning, precise target volume definition, and adequate control of the breathing motion [16]. In the context of locally advanced lung cancer radiotherapy and complex radiation volumes with significant respiratory motion, the addition of various geometric margins leads to the irradiation of a larger volume of healthy tissue, consequently increasing the risk of complications while reducing the possibility of dose escalation [17]. Incorporating tumor motion in the radiotherapy process in order to increase effective tumor targeting and allow a reduction in the planning target volume is a very important issue [18]. In principle, if imaging and treatment are synchronized with the patient's respiratory cycle, there is the potential for CTV-PTV margin reduction [19].

Different retrospective studies have previously evaluated the significance of 4D CT simulation in radical lung cancer radiotherapy treatment. Ahmed et al. [19] made an evaluation to find out if the motion assessment with 4D CT simulation improved the target coverage in lung cancer radiotherapy and showed superior coverage of the target volume compared to 3D simulation. IGTV 4D was significantly larger than GTV 3D for both primary and nodal diseases, either combined or separately. The results of our study are comparable with the above-mentioned results – volumetric value IGTV 4D expressed in cm^3 and ESD IGTV 4D expressed in cm were significantly larger than GTV 3D. This result is expected, given that IGTV 4D is combined after delineating GTV in ten breathing phases, while GTV 3D is delineated only in the FB phase. Correlating lung tumor location and motion with respiration using a 4D CT scan was evaluated by Siow and Lim [20], who emphasized the importance of motion mitigation strategies.

A further evaluation made for this study showed a statistically significant difference between PTV 3D vs. IPTV 4D. The volumetric values and equivalent spherical diameter of PTV were significantly reduced in 4D simulation-based delineation in this study. PTV 3D in both cases was significantly larger than IPTV 4D. That is a very significant result. It is essential to minimize the exposed planning target volume in patients with locally advanced lung cancer and large tumor volume because normal lungs and organs at risk are exposed at large radiation volumes and, consequently, higher doses. Various authors suggest that incorporating tumor motion into the simulation, planning, and delivery is necessary for effective tumor targeting, which provides the possibility of reducing PTV and decreasing the dose to organs at risk [21, 22]. Secondary analysis of the RTOG 0617 study demonstrated that IMRT was associated with lower rates of severe pneumonitis and cardiac doses, thereby justifying the use of IMRT for locally advanced non-small cell lung cancer, which improves the target coverage while minimizing radiation to surrounding tissues [23]. Ueyama et al. [24] suggest that both large PTV volume and large PTV/total lung ratio were significantly associated with radiation pneumonitis.

In this study, 3D-based PTV/total lung ratio vs. 4D-based IPTV/total lung ratio expressed in percentages indicated that 3D-based PTV had a significantly larger volume. That is a burning issue; it is extremely important to decrease radiation volume in order to avoid acute and late side effects to surrounding organs at risk. Clinical studies have shown that minimizing the lung volume irradiated even to extremely low doses can result in fewer pulmonary complications [25]. Matsuo et al. [26] reported that large PTV was a significant risk factor for symptomatic radiation pneumonitis. The main disadvantage of the current study is the small number of patients. However, based on these results, this study indicates the benefits of using 4D simulation over 3D simulation-based delineation and the possibility of decreasing definitive PTV.

Given the respiration in various locations of the lungs, tumor motion has already been widely described. A study conducted by Seppenwoolde et al. [27] used gold fiducial markers inserted into tumors and tracked their motion using a real-time tracking system. They concluded that the largest motion was seen in the cranial-caudal direction in the lower lobe tumors near the diaphragm. Another study evaluated lung tumor motion on a large sample. After analyzing more than 500 hours of data, the highest rates of motion amplitudes, intrafraction/interfraction variation, and tumor baseline changes were in the SI direction (6.0 ± 2.2 mm, 2.2 ± 1.8 mm, 1.1 ± 0.9 mm, and -0.1 ± 2.6 mm) [28]. Motion amplitudes >15 mm were observed only in the lower geometric quarter of the lungs.

The results of this study are compatible with the results acquired in previous studies of lung tumor motion. There was almost no dislocation of the tumor along the X and Y-axes when assessed in the phases GTV 0, GTV 50, GTV FB, or GTV MIP. Unlike the movement along the X and Y-axes, dislocation along the Z-axis (superoinferior) was observed in almost half of the studied cases. When different breathing phases were compared, no statistically significant difference was identified in the movement along the X-axis. Similar results were acquired by the analysis of movement along the Y-axis, i.e., there was no statistically significant difference. The analysis of dislocation along the Z-axis indicated that the difference between the studied phases was highly significant.

An obvious advantage of 4D CT is the possibility of synchronizing the radiation beam with the moving target, and hence it is an important segment of modern and precise radiotherapy. A possible adverse aspect of the 4D CT simulation-based radiotherapy approach is the additional infrastructure required for obtaining 4D CT, personal training, and education of patients, as well as the increased workload for radiation oncologists in the delineation process, compared to 3D simulation-based radiotherapy.

Conclusion

Radiotherapy treatment of lung cancer has entered the era of precision medicine and radiotherapy. Determination of the target area is the key point in radiotherapy.

With 4D CT, personalized approach with individualized patient's margins that encompasses patients breathing motion becomes possible. This study suggests that 4D simulation is an important issue in lung cancer radiotherapy that poses high precision treatment with possibly reduced planning target volume and consequently reduced side effects of radiotherapy.

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Trabeculectomy with mitomycin C for glaucoma secondary to emulsified silicone oil after *pars plana* vitrectomy: a three-year follow-up

Trabekulektomija sa mitomicinom C kod sekundarnog glaukoma nakon *pars plana* vitrektomije sa emulzifikovanim silikonskim uljem: tri godine praćenja

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Abstract

Background/Aim. Different surgical interventions have been proposed, including trabeculectomy associated with antiproliferative agents because silicone oil (SO) removal cannot necessarily provide intraocular pressure (IOP) control. The aim of the study was to determine the efficacy of trabeculectomy with mitomycin C (MMC) for lowering IOP in patients with open-angle glaucoma (OAG) secondary to emulsified SO after *pars plana* vitrectomy. **Methods.** A single-center, prospective study was conducted, from December 2014 to December 2019, on 56 consecutive patients with an uncontrolled elevation of IOP after SO removal who were subjected to trabeculectomy with mitomycin MMC in that period. The primary end-point was the IOP at the three-year follow-up visit. Complete surgical success was defined as an IOP ranging from 7 mmHg to 18 mmHg without glaucoma medication. Qualified success was defined as IOP ≤ 21 mmHg with one or two topical medications. **Results.** Fifty-six patients with a mean age of 53.6 [standard deviation (SD)15.5] years had a mean baseline IOP of 42.3 (39.3 to 45.3) mmHg, which reduced to 18.6 (17.9 to 19.3) mmHg three years after surgery ($p < 0.0001$). Seventeen (30.4%) eyes were classified as a complete success, 21 (37,5%) as a qualified success, and 18 (32.1%) as a failure. In all successfully treated patients, the number of antiglaucoma medications was significantly reduced from 2.85 (SD 0.77) to 1.63 (SD 0.62), $p < 0.0001$. **Conclusion.** Trabeculectomy with MMC may be an option for lowering IOP in patients with OAG secondary to emulsified SO after *pars plana* vitrectomy, which was not controlled with maximum antiglaucomatous medical treatment.

Key words:
glaucoma, open-angle; mitomycin; ophthalmologic surgical procedures; silicone oils; trabeculectomy.

Apstrakt

Uvod/Cilj. Uklanjanje silikonskog ulja (SU) može biti povezano sa nepotpunom kontrolom intraokularnog pritiska (IOP), zbog čega su predložene različite hirurške intervencije, uključujući i trabekulektomiju primenom antiproliferativnih agenasa. Cilj rada bio je da se proceni efikasnost trabekulektomije sa mitomicinom C (MMC) u snižavanju IOP kod bolesnika sa sekundarnim glaukomom otvorenog ugla (OAG) nakon *pars plana* vitrektomije sa SU. **Metode**. Istraživanje je sprovedeno kao jednocentrična, prospektivna studija, od decembra 2014. do decembra 2019. godine, na 56 bolesnika sa nekontrolisanim povišenim IOP, nakon uklanjanja SU, koji su u tom periodu bili podvrgniti trabekulektomiji sa MMC. Krajnji ishod bio je IOP na kontrolnom pregledu nakon tri godine. Kompletan hirurški uspeh definisan je kao IOP u rasponu od 7 mmHg do 18 mmHg, bez lekova koji se primenjuju u lečenju glaukoma. Kvalifikovani uspeh definisan je kao IOP ≤ 21 mmHg, postignut uz primenu jednog ili dva topikalna leka. **Rezultati.** Ukupno 56 bolesnika prosečnog životnog doba od 53,6 [standardna devijacija (SD)15,5] godina imalo je srednji početni IOP od 42,3 (39,3 do 45,3) mmHg, koji se smanjio na 18,6 (od

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17,9 do 19,3) mmHg, 3 godine nakon operacije (p < 0,0001). Kao potpuni uspeh klasifikovano je 17 (30,4%) očiju, kao kvalifikovani uspeh 21 (37,5%) očiju, a 18 (32,1%) očiju klasifikovano je kao neuspeh. Kod svih uspešno operisanih bolesnika broj lekova protiv glaukoma bio je značajno smanjen, sa 2,85 (SD 0,77) na 1,63 (SD 0,62), p < 0,0001. **Zaključak.** Trabekulektomija sa MMC može biti opcija za snižavanje IOP kod bolesnika sa sekundarnim OAG nakon *pars plana* vitrektomije sa emulzifikovanim SU, koji nije bio kontrolisan maksimalnom medikamentnom terapijom protiv glaukoma.

Ključne reči:
glaukom, otvorenog ugla; mitomicini; hirurgija, oftalmološka, procedure; ulja, slikonska; trabekulektomija.

Introduction

Pars plana vitrectomy, combined with artificial vitreous substitutes, is an important treatment for severe retinal detachment (RD) caused by various retinopathies [1, 2]. Among the different vitreous substitutes, silicone oil (SO), introduced by Cibis et al. [3] in 1962, has been widely used in the treatment of complex vitreoretinal diseases over the past several decades [1, 2, 4–6]. However, its ocular tolerance has been repeatedly questioned.

Many different intraocular complications associated with the SO have been previously reported [7, 8]. Among them, the elevation of the intraocular pressure (IOP) is one of the most common complications of SO [7, 9]. Different etiopathogenic mechanisms have been associated with secondary glaucoma to SO, including pupillary block, inflammation, synechial angle closure, rubeosis iridis, and migration of emulsified or non-emulsified SO into the anterior chamber [10, 11].

Although the first therapeutic approach is medical and can include topical or systemic aqueous humor suppressants, surgical management is required if the medical therapy fails to control IOP. However, the optimal surgical intervention for controlling the elevated IOP has not been established.

Since SO removal cannot necessarily provide IOP control, other surgical interventions have been proposed, including trabeculectomy associated with antiproliferative agents, transscleral cyclophotocoagulation, or glaucoma drainage devices which could represent valuable treatment options [12–14]. However, trabeculectomy with mitomycin C (MMC) seems to be associated with lower success rates, as compared with other techniques, mainly due to alterations of the conjunctiva from prior vitreoretinal procedures [11, 14, 15]. In a prospective and randomized study by Errico et al. [12] conducted on patients with ocular hypertension after *pars plana* vitrectomy and SO, who underwent trabeculectomy, a complete and qualified success rate of 40% and 60% of eyes, respectively, was observed.

Similarly, El-Saied and Abdelhakim [14], in a prospective comparative study, reported a success rate with trabeculectomy of 50%, while the success rate with the Ahmed valve was 80%. However, Singh et al. [15], in a prospective study that included 19 patients who underwent trabeculectomy with MMC for glaucoma after vitreoretinal surgery, found that the total success rate was 36.9% at the end of one year, whereas the absolute success rate was only 15.8%. The differences in success rates may be due to the characteristics of the study population or the surgical technique.

The aim of this study was to assess the efficacy of trabeculectomy with MMC for lowering IOP in a cohort of Caucasian patients with OAG secondary to emulsified SO after *pars plana* vitrectomy.

Methods

A single-center prospective interventional case series study was conducted from December 2014 to December 2019 on 56 consecutive patients with uncontrolled elevation of IOP after SO removal, recruited or referred to the University Eye Clinic, Clinical Center of Serbia in Belgrade, previously operated on the territory of the whole Serbia, counting our and three other university clinics and private clinics.

The study protocol was approved by the institutional review board of the University Eye Clinic, Clinical Center of Serbia. All patients were fully informed about the details of the study, and patients provided written informed consent at the beginning of the study. The ethical principles outlined in the Declaration of Helsinki and Good Clinical Practice were followed.

Inclusion criteria were the following: eligible patients were aged 18 or older with an IOP ≥ 21mmHg on maximum antiglaucomatous medical treatment, defined as 3 drugs in two bottles plus oral carbonic anhydrase inhibitors (if not topically administered); SO droplets in the anterior chamber (slit lamp) or anterior chamber angle (gonioscopy) at the time of removal; an attached retina after removal; an open anterior chamber angle.

Exclusion criteria were as follows: any type of angle closure glaucoma; an elevated IOP level attributed to previous vitreoretinal surgery except emulsified SO, such as a scleral buckling procedure; previous laser or surgical glaucoma interventions.

The following data were recorded: demographic, visual acuity (VA), number of glaucoma medications, underlying retinal pathologic findings that required vitreoretinal surgery with SO injection, and status of the lens and anterior chamber angle.

A preoperative evaluation was performed, including measurement of best corrected VA (BCVA) *via* Snellen chart, measurement of IOP *via* Goldmann applanation tonometry, gonioscopy, slit lamp biomicroscopy, and stereoscopic optic disc evaluation with a 90-diopter lens. Dilated fundus examination with binocular indirect ophthalmoscopy was performed between the third and seventh day prior to trabeculectomy.

Surgical technique

Trabeculectomy was performed at least two months after SO removal.

All surgeries were performed under topical anesthesia by a single surgeon who used the same technique. Fornix-based conjunctival dissection was performed at the superior quadrant, supported by 8-0 nylon corneal traction suture. A 15-degree knife was used to delineate, and a crescent knife to create a half-thickness, 4 × 5 mm, rectangular-shaped scleral flap. In all eyes, for a duration of 3 min, MMC 0.04% (0.4 mg/mL) soaked Weck-Cel sponges were applied in Tenon's capsule pocket and also under the sclera flap. After the removal of the sponges, the surgical area was rinsed with 30 mL buffered saline solution. A corneal paracentesis was performed with a 15-degree knife without injecting a viscoelastic solution into the anterior chamber. Sclerectomy was done with a new 15-degree knife, and a peripheral iridectomy was performed in all cases [16]. The scleral flap was sutured with three interrupted 10-0 nylon sutures, and the conjunctiva was sutured with two interrupted 10-0 nylon wing sutures. After the operation, a topical fixed combination of dexamethasone and tobramycin was instilled 4 times daily for 4 weeks, and cyclopentolate 1% one drop twice daily for 2 weeks.

Follow-up evaluation and outcome measures

Postoperative data were collected on days 1 and 7 and months 1, 3, 6, and every 6 months afterward until month 36 of follow-up.

The primary outcome measure was the IOP. According to the Errico et al. [12] criteria, complete surgical success was defined as IOP ranging from 7 mmHg to 18 mmHg without glaucoma medication. Qualified success was defined as IOP ≤ 21 mmHg with one or two topical medications. Those eyes with an IOP > 22 mmHg or an IOP ≤ 21 with more than two glaucoma medications or with the need for additional glaucoma surgery or sight-threatening complications were considered surgical failure.

Postoperative VA was not statistically monitored due to large variations over time, taking into account changes from diabetic retinopathy, the frequency of secondary cataracts, changes in visual field reports, etc.

Statistical analysis

Before the study, it was determined that a sample of at least 53 patients was required to detect a difference of 4 mm Hg in mean IOP at a significance level of 0.05, with a power of 0.90, assuming a standard deviation (SD) of 8 mm Hg. A follow-up loss rate of 20% has been estimated.

A standard statistical analysis was performed using MedCalc Statistical Software version 18.11 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018). Data are expressed as number (percentage), mean (SD), mean [95% confidence interval (95% CI)], or median (95% CI) as appropriate.

Comparisons between preintervention and postintervention values were performed for IOP, a number of antiglaucoma medications, and BCVA.

We analyzed distribution using a D'Agostino-Pearson test. If data were normally distributed, repeated measures ANOVA and the Greenhouse-Geisser correction were used for determining the changes in IOP, the number of antiglaucoma medications, and BCVA. A linear mixed model in order to consider the correlations between the repeated measures and the existence of missing data was used. If data were not normally distributed, the comparisons of the changes in IOP and the number of antiglaucoma medications were performed using the Friedman's two-way analysis test.

Intent-to-treat (ITT) efficacy analyses included all patients who underwent surgery and had at least a valid month one visit.

Per protocol (PP) analyses, which excluded patients who did not complete the study (month 36 visit) or who had major protocol violations, were also conducted to confirm the ITT results.

As required, categorical variables were compared using the $\chi 2$ and Fisher's exact tests.

A p-value < 0.05 was considered statistically significant.

Results

A total of 56 eyes of 56 patients were included in this study, 33 (58.9%) male and 23 (41.1%) female. The mean age was 53.6 (SD 15.5) years, ranging from 18 to 92. Among the study patients, 14 were aphakic and 42 were pseudophakic. At baseline, the mean BCVA was 0.39 (SD 0.26). The main demographic and clinical characteristics of the intent-to-treat (ITT) population are shown in Table 1.

In the ITT population, the mean [95% confidence interval (CI)] baseline IOP was significantly decreased from 42.3 (39.3 to 45.3) mmHg to 18.6 (17.9 to 19.3) mmHg at month 36, $p < 0.0001$ (repeated measures ANOVA and the Greenhouse-Geisser correction) (Figure 1).

Fourteen patients underwent new trabeculectomies with MMC between months 3 and 9 after the first surgery and were considered a failure.

In the per-protocol (PP) study population population, mean (95% CI) baseline IOP was significantly decreased from 41.4 (38.0 to 44.7) mmHg to 19.0 (18.3 to 19.7) mmHg at month 36, $p < 0.0001$ (repeated measures ANOVA and the Greenhouse-Geisser correction) (Figure 2).

After three years of follow-up, 17 (30.4%) eyes were classified as a complete success, 21 (37.5%) as a qualified success, and 18 (32.1%) as a failure.

In the ITT population, the number of antiglaucoma medications was significantly reduced from 2.85 (0.77) to 1.63 (0.62), $p < 0.0001$.

In the ITT population, the values of IOP, VA, and the number of antiglaucoma medications for all three groups are shown in Table 2.

Table 1

Baseline demographic and clinical characteristics of the intent to treat (ITT) study population

Variable	Values
Age (years), mean (SD), 95% CI	53.6 (15.5), 49.4 to 57.7
Sex (man/woman), n (%)	33 (58.9)/23 (41.1)
IOP (mm Hg), mean (SD), 95% CI	42.3 (10.6), 39.3 to 45.3
BCVA, mean (SD), 95% CI	0.39 (0.26), 0.32 to 0.46
Mean defect (dB), mean (SD), 95% CI	-8.0 (2.3), -9.3 to -6.8
Cup-to-disk ratio, mean (SD), 95% CI	0.47 (0.22), 0.41 to 0.52
Antiglaucoma medication, n mean (SD), 95% CI	2.85 (0.77), 2.31 to 3.19

SD – standard deviation; CI – confidence interval; n – number; IOP – intraocular pressure; BCVA – best corrected visual acuity; dB – decibels.

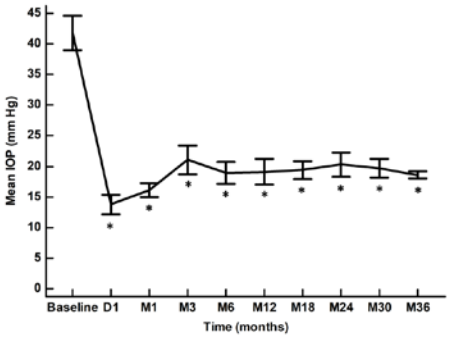

Fig. 1 – Mean intraocular pressure (IOP) over the course of follow-up in the intent to treat (ITT) study population. The vertical bars represent the 95% confidence interval.
***p < 0.001 as compared to baseline (repeated measures ANOVA and the Greenhouse-Geisser correction).**

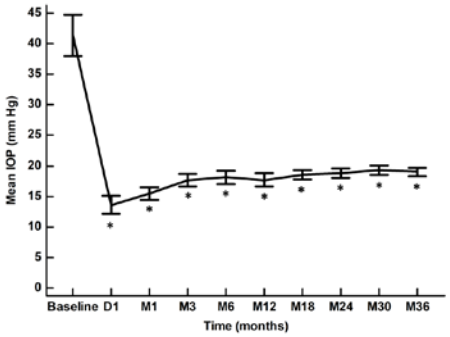

Fig. 2 – Mean intraocular pressure (IOP) over the course of follow-up in the per-protocol study population. The vertical bars represent the 95% confidence interval.
***p < 0.001 as compared to baseline (repeated measures ANOVA and the Greenhouse-Geisser correction).**

Table 2

Overview of the values of intraocular pressure (IOP), visual acuity, and number of antiglaucoma medications and their changes to baseline in the intent to treat (ITT), complete success, qualified success, and failure populations

Variable	ITT (patients, n = 56)	*p**	Complete success (patients, n = 17)	*p* **	Qualified success (patients, n = 21)	*p* **	Failure‡ (patients, n = 18)	*p* **
IOP	-42.3 (-45.3 to 39.3)	< 0.0001	-13.8 (-17.9 to -7.0)	< 0.0001	-15.6 (-21.0 to -12.3)	< 0.0001	-25.1 (-29.9 to -19.2)	< 0.0001
BCVA	-0.03 (-0.08 to 0.02)	0.1851	-0.05 (-0.14 to 0.04)	0.2620	-0.01 (-0.11 to 0.13)	0.8481	-0.07 (-0.10 to -0.05)	< 0.0001
Antiglaucoma medications, n	-2.85 (-3.52 to 2.13)	< 0.0001	0	/	-1.5 (-2.0 to -1.0)	0.8123	-2.62 (-3.24 to 2.05)	0.0977

All values are expressed as mean (95% CI) difference from baseline.
n – number; CI – confidence interval; IOP – intraocular pressure; BCVA – best corrected visual acuity.
***repeated measures ANOVA and the Greenhouse-Geisser correction; ****Friedman test; ‡mean difference was calculated comparing the baseline IOP of the first surgery with the last IOP measurement of the second trabeculectomy.**

Besides the fourteen patients requiring additional filtration surgeries, two patients had a choroidal abscess and one had an vitreous hemorrhage (required medical treatment). None of the patients developed hypotony.

Discussion

The results of this study, partly previously presented at congress [17], found that trabeculectomy with MMC

effectively reduces the elevated IOP in patients with open-angle glaucoma secondary to emulsified SO after *pars plana* vitrectomy. Although the rate of complete success was relatively low and lower than reported for most refractory glaucoma, it was in line with the scientific evidence.

In line with our results, Errico et al. [12] found in their study that complete success was achieved in 40% of eyes that underwent trabeculectomy, using the same criteria for complete and qualified success. However, comparing our results to those of Errico et al. [12] is difficult because the follow-up period of our study was 36 months, while the one in the Errico et al. [12] study was 24 months.

The results of this study are much better than those reported by Singh et al. [15], who reported a complete success rate of 15.8% in a prospective study conducted on patients who underwent trabeculectomy with MMC for glaucoma after vitreoretinal surgery.

However, the success rate found in our study is slightly lower than that observed by El-Saied and Abdelhakim [14], who, in a prospective study, compared the outcome of four different surgical procedures, namely trabeculectomy, deep sclerectomy, Ahmed valve, and Ex-Press Minishunt. The success rate in the trabeculectomy group in their study was 50%, slightly greater than that observed in our study (30.4%).

When comparing the outcomes of glaucoma surgery, it is essential to bear in mind that the surgical technique may have variations among surgeons and may have different results depending on the study population (race, age, etc.).

Therefore, the different success rates may be partially explained by the existence of variable study designs, treatment periods, and ethnic populations.

The success rate in our study is much lower than that in other types of glaucoma [18, 19].

The high failure rate of trabeculectomy, either with or without antimetabolites, may be due to different factors, including conjunctival scarring from the vitreoretinal surgery, the presence of macrophages, and an inflammatory reaction in the internal ostium [6, 7].

Although the tubes have shown better results, it is noteworthy mentioning that the treatment of this type of glaucoma must be customized [13, 14, 20]. However, it should be mentioned that although drainage implants are an alternative surgical option, oil migration can occur through the tube into the subconjunctival space inciting an inflammatory reaction [21].

Regarding tolerability, our study suggested that trabeculectomy with MMC was, on average, well tolerated in these patients.

This study has inherent limitations concerning the interpretation of its results as it is an open-label, non-randomized, non-controlled study by design, and caution needs to be employed while deriving conclusions. Nevertheless, the sample size was calculated prior to the study.

Conclusion

The results of this study suggested that trabeculectomy with MMC may be an option for treating OAG glaucoma secondary to SO, although its success rate was relatively low.

However, the study design does not allow us to reach decisive conclusions on the comparative efficacy and safety of trabeculectomy with MMC in such patients.

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Costs and consumption of analgesics, with special reference to opiates in Serbia and Montenegro from 2015 to 2019

Troškovi i potrošnja analgetika, sa specijalnim osvrtom na opijate u Srbiji i Crnoj Gori od 2015. do 2019.

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Abstract

Background/Aim. Patients in developing countries do not always receive adequate pain-relieving treatment. Monitoring analgesics consumption is of great importance since this can help assess the quality of painful condition management. The aim of this paper was to present a five-year consumption and costs of drugs with analgesic effects in developing countries, exemplified by Serbia and Montenegro, and indicate the main reasons for their (in)adequate prescribing. **Methods.** The observational, retrospective, cross-sectional study was conducted in order to analyze the consumption of all analgesics, both opioid and non-opioid, in Serbia and Montenegro, as developing countries. The data concerning analgesic consumption and drug prices were obtained from annual editions of the publications of the Medicines and Medical Devices Agency of Serbia and Montenegro. The World Health Organization methodology with defined daily dose (DDD) as a unit of measure (defined by the number of DDD per 1000 inhabitants per day) was used in these publications. **Results**. Over the course of five years (from 2015 to 2019) in Serbia, the total allocations for analgesic therapy had a rising trend, from about 43.6 million to 63.3 million euros, while in Montenegro, expenditures showed annual variations with the highest value in 2018. Most of the money in both countries was invested in the M01A group of drugs, for which the highest consumption was also recorded. Significantly higher consumption of opioid analgesics in Montenegro compared with Serbia was observed in the same period, and it predominantly reflected the difference in fentanyl (N02AB03, transdermal patch) prescribing. In Montenegro, consumption of the M01AB group of drugs was prominently higher in comparison to the M01AE drugs group during the whole five-year period, like in Serbia, in which this was not the case just in 2018. **Conclusion**. Taking into account the importance of analgesics for everyday medical practice, more rational prescribing of these drugs is necessary both in Serbia and Montenegro in the future.

Key words:
analgesics; analgesics, opioids; developing countries; drug prescriptions; montenegro; pain; serbia.

Apstrakt

Uvod/Cilj. Pacijenti u zemljama u razvoju nemaju uvek adekvatan tretman za ublažavanje bolova. Praćenje potrošnje analgetika je od velike važnosti, jer može pomoći u proceni kvaliteta upravljanja terapijom bolnih stanja. Cilj rada bio je da predstavi petogodišnju potrošnju i troškove lekova sa analgetskim dejstvom u zemljama u razvoju, na primerima Srbije i Crne Gore, i ukaže na glavne razloge njihovog (ne)adekvatnog propisivanja. **Metode.** Opservaciona, retrospektivna studija preseka sprovedena je kako bi se analizirala potrošnja svih analgetika, opioidnih i neopioidnih, u Srbiji i Crnoj Gori kao zemljama u razvoju. Podaci koji se odnose na potrošnju lekova i nastale troškove korišćeni su iz publikacija koje svake godine izdaju naconalne agencije za lekove i medicinska sredstva Srbije i Crne Gore. U okviru ovih publikacija korišćena je metodologija Svetske zdravstvene organizacije sa definisanom dnevnom dozom (DDD) kao jedinicom mere (broj DDD na 1000 stanovnika na dan). **Rezultati.** Tokom petogodišnjeg perioda (od 2015. do 2019. godine) ukupna izdvajanja za terapiju analgeticima u Srbiji pokazala su trend porasta (od oko 43,6 miliona na 63,3 miliona evra), dok su troškovi u Crnoj Gori varirali na godišnjem nivou, sa najvišim vrednostima 2018. godine. Najveći deo sredstava u obe zemlje bio je investiran u lekove grupe M01A, za koje je

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ujedno pokazana i najveća potrošnja. Značajno viša potrošnja opioidnih analgetika u Crnoj Gori, u poređenju sa Srbijom, zapažena je u ovom periodu i ona prevashodno odražava razlike u propisivanju fentanila (N02AB03, transdermalni flaster). U Crnoj Gori potrošnja lekova iz grupe M01AB bila je značajno viša u poređenju sa potrošnjom iz grupe M01AE u toku celog petogodišnjeg perioda, slično kao i u Srbiji, gde to nije bio slučaj samo u 2018. godini. **Zaključak.** Uzimajući u obzir značaj analgetika kao grupe lekova za svakodnevnu lekarsku praksu, neophodno je njihovo racionalnije propisivanje i u Srbiji i u Crnoj Gori u budućem periodu.

Ključne reči:
analgetici; analgetici, opioidni; zemlje u razvoju; lekovi, propisivanje; crna gora; bol; srbija.

Introduction

Although pain management is one of the fundamental human rights [1], patients in developing countries do not always receive adequate pain-relieving treatment. It has been estimated that about 5.5 billion people, or 80% of the global population, have limited or no access to pain management [2]. Those are mainly low-income countries (LIC) or low-middle-income countries (LMIC).

Although access to pain management is low in many underdeveloped countries, it is believed that there is a "treatment gap" between what is being done and what could be done [3]. Barriers to the provision of effective pain management in developing countries are quite diverse. Most commonly, references are made to legislative regulations relating to medicines, i.e., accessibility and availability of some drugs, cumbersome medicine distribution process, and limited daily consumption of opioids, regardless of individual patient's needs [2–4]. It has been estimated that only 6.7% of the total consumption of natural opioid morphine is consumed by 74% of the global population in which the cancer-related mortality rate is the highest [5]. Existing obstacles can be related to health professionals that are overburdened by work and improperly trained in pain management, and frequently laden with prejudices relating to prescribing and administering opioids. There is also a fear of addiction to using opioids in the patients, frequently referred to as "opiophobia", but also a fear of respiratory depression and other serious adverse effects of this therapy [6]. On the other hand, patients also have prejudices. Many of them, particularly in underdeveloped countries, believe that complaining of pain is undignified, i.e., that pain is something to be endured. In some cultures and religions, it is even believed that pain is a "ticket to heaven" [7].

Whatever the reasons, overcoming these obstacles to pain management is crucial in developing countries. That can be achieved by harmonization and amendments to the laws, increased access to medicines, and their more affordable prices. Furthermore, education of both health professionals and the general population on the fundamental human right to pain relief and the administration of appropriate therapy has to be promoted.

Monitoring analgesic consumption is of great importance, particularly opioids, since this can help assess the quality of painful condition management. Moreover, consumption of non-opioid analgesics in many countries is significantly higher than in other groups of drugs, and one of the explanations is that many representatives from this group are available as over-the-counter (OTC) drugs. Therefore, they are among the best-selling drugs. However, due to their status, there is more and more evidence linking non-steroidal anti-inflammatory drugs (NSAIDs) and different side-effect profiles and their possible negative influence on human health [8].

It is expected that analgesics consumption will rise in the following years, and the reasons may vary as follows: higher rate of traffic-related injuries, wars, or terrorist actions, higher rate of patients with various comorbidities due to population aging, higher rate of surgical procedures, chronic painful conditions, and other [9]. Although there are many cheap, safe, and effective drugs on the market, pain management remains inadequate in numerous healthcare systems [10–13].

The aim of this paper was to present a five-year consumption and costs of drugs with analgesic effects, especially opioids, in developing countries, exemplified by Serbia and Montenegro, and indicate the main reasons for their (in)adequate prescribing.

Methods

The retrospective cross-sectional observational study was conducted in order to analyze consumption of all analgesics, both opioid and non-opioid, in Serbia and Montenegro as developing countries [The World Bank classifies countries by personal income into four groups: LIC, LMIC, upper-middle-income countries – UMIC, and high-income countries – HIC, depending on the gross national product (GDP) per capita] [14]. According to this classification, Serbia and Montenegro, with 7,030 USD and 9,060 USD GDP per capita, respectively, are classified as UMIC.

The data concerning analgesic consumption and drug prices from 2015 to 2019 in Serbia and Montenegro were directly obtained from editions of the publication "Marketing and Consumption of Medicinal Products in Human Medicine", issued annually by the Medicines and Medical Devices Agency of Serbia and the publication titled "Consumption of Medicines in Montenegro", issued by the Institute for Medicines and Medical Devices of Montenegro.

These data from the above-mentioned agency publications were expressed by the World Health Organization (WHO) methodology with a defined daily dose (DDD) as a unit of measure [15]. The method used to present the consumption of analgesics is determined by the number of DDD per 1000 inhabitants per day (DDD/1000 inhabitants/day) [16, 17].

Results were obtained for the following groups of analgesics, according to the Anatomical Therapeutic Chemical (ATC) codes: NO2A – opioids; N02AA – natural opium alkaloids (N02AA01 morphine, N02AA03 hydromorphone, N02AA05 oxycodone, N02AA55 oxycodone and naloxone); N02AB – phenylpiperidine derivatives (N02AB02 pethidine, N02AB03 fentanyl); N02AJ – opioids in combination with non-opioid analgesics (N02AJ13 tramadol and paracetamol); N02AX – other opioids (N02AX02 tramadol); NO2B – other analgesics and antipyretics; N02BA – salicylic acid and derivatives (N02BA01 acetylsalicylic acid); N02BB pyrazolones (N02BB02 metamizole sodium); N02BE anilides (N02BE01 paracetamol); N02C – antimigraine preparations (N02CA ergot alkaloids, N02CA52 ergotamine, combinations excluding psycholeptics); N02CC – selective serotonin (5HT1) agonists (N02CC01 sumatriptan, N02CC03 zolmitriptan, N02CC07 frovatriptan); M01A – anti-inflammatory and antirheumatic products, non-steroids; M01AB – acetic acid derivatives and related substances (M01AB05 diclofenac, M01AB08 etodolac, M01AB11 acemetacin, M01AB15 ketorolac, M01AB16 aceclofenac, M01AB55 diclofenac, combinations); M01AC – oxicams (M01AC01 piroxicam, M01AC05 lornoxicam, M01AC06 meloxicam); M01AE – propionic acid derivatives (M01AE01 ibuprofen, M01AE02 naproxen, M01AE03 ketoprofen, M01AE09 flurbiprofen, M01AE17 dexketoprofen, M01AE51 ibuprofen, combinations); M01AH – coxibs (M01AH01 celecoxib, M01AH05 etoricoxib); M01AX – other anti-inflammatory and antirheumatic agents, non-steroids (M01AX17 nimesulide) [16]. Costs of analgesic treatment expressed in euros (EUR) are also presented from cited agency publications.

Results

Over the five years covered by this observational study in Serbia, the total allocations for all medicines had a rising trend – from 851,476,036 in 2015 to 1,176 million EUR in 2019 (Figure 1). The situation is similar to drugs with analgesic effects which also showed a rising trend, from about 43.6 to 63.3 million EUR. If the costs of analgesic therapy are expressed as a percentage of total expenditures for medicines, it can be concluded that about 5.5% (from 5.12% in 2015 to 5.68% in 2017) of the total sum accounts for the consumption of analgesics. Most of the money spent on analgesics was invested in the M01A group; the same result was obtained if DDD/1000 inhabitants/day was used as a consumption indicator (Tables 1 and 2).

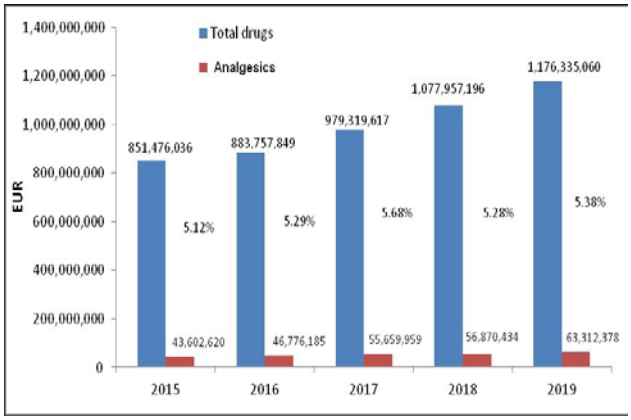

Fig. 1 – Total costs for medications as well as drugs with analgesic effects in Serbia during the five-year period (2015–2019).

Table 1

Expenditures and consumption of the main groups of analgesics, defined by ATC classification, in Serbia during the five-year period (2015-2019)

Analgesics	2015		2016		2017		2018		2019	
	EUR	DDD	EUR	DDD	EUR	DDD	EUR	DDD	EUR	DDD
N02A opioids	2,290,093	0.5630	2,245,910	0.5677	2,172,640	0.5244	2,205,030	0.5508	2,292,328	0.5683
N02B other analgesics and antipyretics	15,502,549	6.0390	19,712,955	7.9546	23,300,050	5.3067	26,603,908	7.0746	28,600,499	7.9010
N02C antimigraine preparations	284,822	0.0870	427,575	0.1606	541,720	0.1829	512,175	0.1575	661,829	0.2451
M01A anti-inflammatory and antirheumatic products, non-steroids	25,525,155	62.7660	24,389,745	58.2578	29,645,549	76.5431	27,549,320	59.0044	31,757,723	69.5517
Total	43,602,619	69.4550	46,776,185	66.9407	55,659,959	82.5571	56,870,433	66.7873	63,312,379	78.2661

ATC – Anatomical Therapeutic Chemical; DDD – defined daily dose; EUR – euro.

Table 2

Expenditures and consumption of analgesics, defined by ATC classification, in Serbia during the five-year period (2015-2019)

Drugs	2015		2016		2017		2018		2019	
	EUR	DDD	EUR	DDD	EUR	DDD	EUR	DDD	EUR	DDD
N02A Opioids										
N02AA Natural opium alkaloids	805,535	0.1070	800,976	0.1018	675,274	0.0822	597,796	0.0815	861,585	0.1289
N02AB Phenylpiperidine derivatives	907,508	0.1130	873,586	0,1100	953,592	0.1178	1,031,318	0.1302	905,092	0.1138
N02AJ Opioids in combination with non-opioid analgesics									12,931	0.0080
N02AX Other opioids	577,050	0.3420	571,348	0.3560	543,774	0.3245	575,917	0.3391	512,720	0.3176
N02B Other analgesics and antipyretics										
N02BA Salicylic acid and derivatives	3,313,884	1.2430	2,365,001	0.7981	2,957,054	0.9986	2,650,783	0.7781	2,258,336	0.7408
N02BB Pyrazolones	1,750,252	1.8450	2,050,210	2.3514	1,954,986	1.8791	1,619,823	1.5569	1,942,372	1.9182
N02BE Anilides	10,438,413	2.9500	15,297,743	4.8050	18,388,010	2.4290	22,333,303	4.7396	24,399,791	5.2421
N02C Antimigraine preparations										
N02CA Ergot alkaloids			50,474	0.0306	87,377	0.0511			128,132	0.0764
N02CC Selective serotonin (5HT1) agonists	284,822	0.0870	377,101	0.1300	454,343	0.1318	512,176	0.1575	533,696	0.1686
M01A Anti-inflammatory and antirheumatic products, non-steroids										
M01AB Acetic acid derivatives and related substances	10,452,839	33,0110	9,214,272	27.2548	13,424,454	42.6273	9,107,148	23.2733	11,305,545	31.1431
M01AC Oxicams	1,556,009	3.3280	1,450,914	3.0859	1,359,410	2.8377	1,225,632	2.6577	1,047,199	2.2840
M01AE Propionic acid derivatives	10,330,280	19.2670	10,714,087	20.3967	12,598,539	23.0325	14,871,261	25.0473	17,133,916	28.3865
M01AH Coxibs	302,855	0.0280	258,876	0.0227	395,185	0.3254	531,460	0.4476	556,567	0.5199
M01AX Other anti-inflammatory and antirheumatic agents, non-steroids	2,883,171	7.1320	2,751596	7.4978	1,867,962	7.7203	1,813,819	7.5785	1,714,496	7.2181

ATC – Anatomical Therapeutic Chemical; DDD – defined daily dose; EUR – euro.

During the same five-year period in Montenegro, the total allocations for medicines also showed a rising trend, from about 66.1 million in 2015 to 101 million EUR in 2019 (Figure 2). The situation is similar with analgesic therapy which also shows a rising trend but with annual variations. In 2018, 1.25 million EUR were spent more than in 2015, while in 2019, a smaller sum (4,220,685) was spent than in the preceding year. The result is substantiated if DDD/1000 inhabitants/day is used as an indicator, and most of the money spent on analgesics was invested in the M01A group, like in Serbia (Tables 3 and 4).

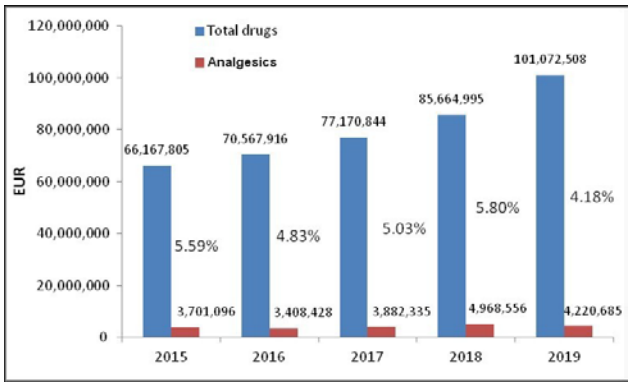

Fig. 2 – Total costs for medications as well as drugs with analgesic effects in Montenegro during the five-year period (2015–2019).

If we compare the use of opioid analgesics between Serbia and Montenegro, significantly higher consumption of these medicines could be observed in Montenegro (Figure 3). In Serbia, their consumption was relatively stable and maintained at about 0.50 DDD/1000 inhabitants/day. On the other hand, in Montenegro, it was significantly higher (0.96 and 1.00 DDD/1000 inhabitants/day in 2015 and 2016, respectively, falling to 0.76 in 2017 and again reaching the previous level in 2018 and 2019 (Figure 3). This difference in use reflects the difference in fentanyl (N02AB03, transdermal patch) prescribing (Table 5). Consumption of opioid fentanyl in Montenegro was 0.8 DDD/1000 inhabitants/day and higher in the observed period, except in 2017, when the value dropped to 0.62. In Serbia, the volume of this consumption was set from 0.11 to 0.12 DDD/1000 inhabitants/day in the same period. However, morphine sulfate consumption was prominently lower in Montenegro than in Serbia during the five years, while hydromorphone, oxycodone, and the combination of oxycodone and naloxone were not present in the Montenegrin market. Consumption of tramadol was also lower in Montenegro compared with Serbia but not as prominently as was the case with morphine sulfate.

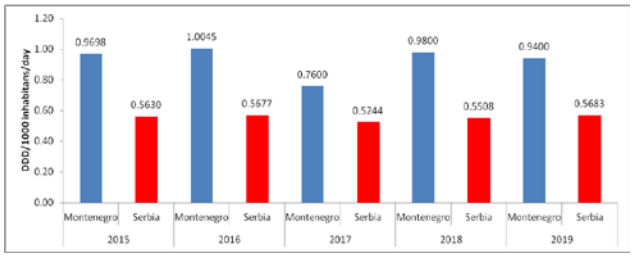

Fig. 3 – Total use of opioids in Serbia and Montenegro during the five-year period (2015–2019). DDD – defined daily dose.

As far as non-opioid analgesics are concerned, consumption of the N02B group of drugs was constantly rising both in Serbia and Montenegro, as well as Serbian expenditures for these drugs (except in 2017) (Tables 1 and 3). As far as the N02BB group is concerned (pyrazolones), only metamizole was present on the market of both countries (Tables 2 and 4). Metamizole costs varied

Table 3

Expenditures and consumption of the main groups of analgesics, defined by ATC classification, in Montenegro during the five-year period (2015-2019)

Analgesics	2015		2016		2017		2018		2019	
	EUR	DDD	EUR	DDD	EUR	DDD	EUR	DDD	EUR	DDD
N02A Opioids	89,115	0.9698	86,776	1.0045	66,411	0.7600	119,747	0.9800	122,164	0.9400
N02B Other analgesics and antipyretics	1,230,236	7.3088	959,075	7.7828	1,337,056	7.5000	835,494	7.5300	1,362,607	8.0700
N02C Antimigraine preparations	61,687	0.0834	62,302	0.1634	63,872	0.1800	67,639	0.1900	72,907	0.2000
M01A anti-inflammatory and antirheumatic products, non-steroids	2,320,058	72.9602	2,300,275	68.4748	2,414,996	74.5400	2,587,188	73.3000	2,663,007	75.3900
Total	3,701,096	81.3222	3,408,428	77.4255	3,882,335	82.9800	3,610,068	82.0000	4,220,685	84.6000

ATC – Anatomical Therapeutic Chemical; DDD – defined daily dose; EUR – euro.

Table 4

Expenditures and consumption of analgesics, defined by ATC classification, in Montenegro during the five-year period (2015-2019)

N02A Opioids	2015		2016		2017		2018		2019	
	EUR	DDD	EUR	DDD	EUR	DDD	EUR	DDD	EUR	DDD
N02AA Natural opium alkaloids	14,385	0.0113	17,464	0.0154	12,555	0.0100	16,695	0.0200	19,884	0.0200
N02AB Phenylpiperidine derivatives	61,476	0.8219	57,705	0.8598	42,115	0.6200	86,783	0.8400	83,198	0.8000
N02AJ Opioids in combination with non-opioid analgesics	774	0.0001	424	0.0001	340	0.0001	194	0.0001	298	0.0001
N02AX Other opioids	12,480	0.1366	11,153	0.1293	11,400	0.1300	16,075	0.1300	18,783	0.1100
N02B Other analgesics and antipyretics										
N02BA Salicylic acid and derivatives	216,585	1.0722	206,116	1.0476	205,107	0.8900	190,712	0.7300	182,574	0.7000
N02BB Pyrazolones	266,991	3.3598	279,268	3,3662	277,539	3.3400	264,644	3.2600	294,762	3.2300
N02BE Anilides	746,660	2.8768	473,691	3.3690	854,409	3.2700	1,290,850	3.5400	885,271	4.1300
N02C antimigraine preparations										
N02CA Ergot alkaloids	34,302	0.1104	37,365	0.1199	42,373	0.1400	43,420	0.1400	42,541	0.1400
N02CC Selective serotonin (5HT1) agonists	27,385	0.0417	24,937	0.0435	21,499	0.0400	24,219	0.0500	30,365	0.0600
M01A Anti-inflammatory and antirheumatic products, non-steroids										
M01AB Acetic acid derivatives and related substances	966,223	45.3044	819,712	39.9800	842,036	44.5500	1,210,354	45.7600	1,290,080	49.7700
M01AC Oxicams	58,073	2.2097	74,272	2.3100	77,554	2.3700	46,805	1.2200	41,565	1.1000
M01AE Propionic acid derivatives	1,150,506	21.2335	1,200,789	22.4459	1,235,481	22.9400	1,219,430	23.4400	1,278,751	23.7400
M01AH Coxibs	13,484	0.1104	18,815	0.1589	23,956	0.2000	27,826	0.2400	30,359	0.2600
M01AX Other anti-inflammatory and antirheumatic agents, non-steroids	131,772	2.6657	186,687	3.5800	235,969	4.4800	82,773	2.6400	22,252	0.5200

ATC – Anatomical Therapeutic Chemical; DDD – defined daily dose; EUR – euro.

Table 5

Consumption of opioids (in DDDs/1000 inhabitants/day) in Serbia and Montenegro during the five-year period (2015-2019)

N02A Opioids	2015		2016		2017		2018		2019	
	Serbia	Montenegro	Serbia	Montenegro	Serbia	Montenegro	Serbia	Montenegro	Serbia	Montenegro
N02AA01 Morphine-sulfate	0.1080	0.0113	0.1018	0.0154	0.0493	0.0100	0.0497	0.0200	0.0616	0.0200
N02AA03 Hydromorphone	0.0490		0.0523		0.0330		0.0269		0.0353	
N02AA05 Oxycodone							0.0048		0.0249	
N02AA55 Oxycodone, naloxone									0.0072	
N02AB02 Pethidine	0.0010		0.0019		0.0023	0.0001	0.0020	0.0001	0.0016	0.0001
N02AB03 Fentanyl	0.1120	0.8219	0.1080	0.8598	0.1155	0.6200	0.1282	0.8400	0.1122	0.8000
N02AJ13 Tramadol, paracetamol						0.0001		0.0001	0.0080	0.0001
N02AX02 Tramadol	0.3420	0.1366	0.3560	0.1293	0.3245	0.1300	0.2971	0.1300	0.3176	0.1100

DDD – defined daily dose.

from year to year in Serbia, but it was higher at the end of the observed period compared to 2015. A similar situation was with its consumption. Neither consumption nor costs for metamizole changed significantly during the entire observed period in Montenegro (Tables 2 and 4). A rising trend of expenditures for paracetamol (N02BE anilides) was noticed in the entire monitored period in Serbia, while it varied from year to year in Montenegro. Paracetamol consumption in Serbia was significantly higher in 2019 compared to 2015, although its lowest value was recorded in 2017, not accompanied by a decrement in cost. A similar situation was in Montenegro, but the lowest value of its consumption was not as prominent as in Serbia in 2017.

Most prominent expenditures and consumption of all analgesics recorded in both Serbia and Montenegro during the whole observed period referred to the M01A group of drugs (Tables 1 and 3). Consumption of the M01AB group of drugs was higher than that of the M01AE group in Serbia during the observed period except in 2018 when it was lower. As far as costs were concerned, they were similar for both groups of drugs from 2015 to 2017, while expenditures for the M01AE group were higher than for the M01AB group in 2018 and 2019. In Montenegro, consumption of the M01AB group of drugs was prominently higher compared to the M01AE group during the whole five-year period. However, expenditures for the M01AE group of drugs were higher from 2015 to 2017, while they were very similar for both groups of drugs during the remaining two years of the observed period. In both countries over the five years, the expenditures and consumption of coxibs were the lowest among other anti-inflammatory drugs.

Discussion

In our study, the trends in analgesic consumption and expenditures in Serbia and Montenegro from 2015–2019 were examined. The total consumption of all drugs on the markets of both countries and their prices increased during this period. The highest overall consumption of analgesics was observed in 2017 in Serbia (82.55 DDD/inhabitants/day), while in Montenegro, it was detected in 2019 (84.60 DDD/inhabitants/day). Although the corresponding consumption decreased in 2016 and 2018, the total costs were steadily rising in Serbia, while in Montenegro, it varied annually, and the highest costs for analgesics were in 2019 when their consumption was also highest. Occasionally observed increase in costs of analgetics parallel with the decline in their consumption, which alternates with the reduction of costs in the supervenient year in Serbia, could be explained by government decrees that seek to limit drug prices in accordance with its other economic measures [18]. In both countries, results showed that the M01A group of drugs had the highest consumption associated with the highest costs of all analyzed analgesics. The second highest consumption was related to the N02B group of drugs (other analgesics and antipyretics), followed by opioids (N02A). About 0.22% of the total expenditures for medicines in Serbia were spent on opioids. Consumption of opioids was substantially lower than the consumption of anti-inflammatory medicines and antipyretics. This trend can be partly explained by the fact that most of the latter ones are in the OTC status, i.e., a doctor's prescription is not required. The comparison of the use of opioids over the five years in two countries has led us to the conclusion that it was consistently and more prominent in Montenegro.

In Serbia, during the observed period, consumption of morphine, hydromorphone, fentanyl transdermal formulation, and tramadol was relatively stable, while pethidine use showed a downward trend. In Montenegro, the consumption of morphine, pethidine, and tramadol was stable and lower than in Serbia during the whole observed period. The increase in whole opioid consumption recorded in Montenegro was actually at the expense of fentanyl, of which usage ranged from 0.62 to 0.84 DDD/1000 inhabitants/day. In the same period, from 2015 to 2019, consumption of fentanyl in Serbia was in the range from 0.1080 to 0.1282, while in Croatia, it accounted for 0.63, 0.61, 0.59, 0.60, and 0.63 DDD/1000 inhabitants/day, respectively [19]. A European study concerning the consumption of opioids in severe pain from 1990 to 2016 showed that in 2016 fentanyl use in Sweden, Denmark, and Norway (countries with well-developed pharmacotherapeutic practice) was 5.10, 6.41, and 5.55 times higher than in Serbia [20]. Trends of fentanyl use largely corresponded to those of total opioids in all European countries, and it steadily increased from 2004 onwards. Easy administration, good adherence of the patient to the drug, and a strong marketing campaign have contributed to the increase in its consumption. It is obvious that its use in Serbia is inadequate and that education of both healthcare providers and patients is urgently needed.

On the Montenegrin market, hydromorphone, oxycodone, or the combination of the latter with naloxone were not available at all. Oxycodone appeared on the Serbian market in 2018, while the combinations of oxycodone and naloxone, as well as tramadol and paracetamol, were launched in 2019. The latter combination has also been

present on the Montenegrin market since 2017. Regardless of the rising trend of opioid use in both countries, their consumption remains low. Our previous results indicated that consumptions of opioids in Serbia were 0.517 and 0.519 in 2012 and 2013, respectively, while morphine use was also low and amounted to 0.031 and 0.068 DDD/1000 inhabitants/day in the same years, respectively [21]. At the same time, total opioid costs amounted to 2,637,364.9 and 2,499,864.2 EUR in 2012 and 2013, compared to 2,290,093, 2,245,910, 2,172,640, 2,205,030, and 2,292,328 EUR in 2015, 2016, 2017, 2018, and 2019, respectively. On the other hand, the consumption of opioids in Croatia was 8.83 and 8.55 times higher than in Serbia in 2012 and 2013, respectively, while the Croatian health system was willing to pay 6.35 and 6.22 times more for these drugs in the same period, respectively [21]. That trend continued since the consumption of opioids was 4.13, 4.25, and 16.20 in 2017, 2018, and 2019 in Croatia, respectively [19]. Therefore, it was still far more than in Serbia and Montenegro in the same period.

One of the explanations is the high level of caution in the prescription routine by medical doctors, bearing in mind possible adverse effects and risk of abuse, especially in primary health care in Serbia [22]. A similar situation also exists in other countries [23]. However, the low consumption rate not only in Serbia and Montenegro but also in some other countries, mostly in Southern and Eastern Europe, can suggest insufficiently developed palliative care and numerous administrative obstacles that may still be related to prejudices concerning opioids, but also lack of adequate training of healthcare professionals, still inadequate available formularies and limited economic resources [24, 25]. Namely, there is a globally widespread inequity in access to analgesics, primarily opioids. In 2020, Duthey and Sholten [26] found that only 7.5% of the world population has moderate or adequate access to analgesics. Almost 2 billion people worldwide have no access to essential medicines, resulting in higher pain and suffering, prolonged disease, unnecessary disability, and avoidable fatal outcomes [27]. That applies mostly to opioids that are faced with an abundance of legal obstacles. Moreover, it was shown that there is no correlation between access to potent analgesics and obstacles in legal regulations in eleven East European countries, suggesting that other factors, beyond legislation and regulation, affect inadequate access to medicines [28]. Even though Serbia and Montenegro belong to the UMIC group, they are in the group of about 75% of countries with middle income and inadequate access to analgesics [29].

Due to its pharmacological properties and low price, morphine should be widely used in treating severe pain, particularly cancer. However, 90% of global morphine consumption is used by only 20% of the global population, particularly in developed countries and the ones with high middle income [30]. Although Serbia and Montenegro are in the group of UMIC, consumption of morphine was very low, about 0.10 DDD/1000/day in 2015 and 2016, after that from 0.04 to 0.06 in Serbia (2017–2019), and from 0.01 to 0.02 DDD/1000/day in Montenegro, in the examined five-year period. In 2016, morphine consumption in Serbia was 12.52, 12.58, and 15.9 times lower than in Sweden, Norway, and the European Union, respectively. At the same time, this difference was even more prominent in the same period concerning Montenegro [20]. In our previous study concerning the utilization of parenteral morphine in the tertiary care hospital in Serbia, a low level of morphine use was demonstrated in comparison to other European countries [31], and one of the reasons was a low marketing price connected with no-brand names. In addition, better education and training of staff and a multidisciplinary approach should enable more rational use of opioids, not only in the hospital.

Problems relating to opioids in developing countries do not pertain to morphine only. It also frequently happens that the prices of opioids in some developing countries are higher than in developed ones [32]. There is a study that shows that the price of morphine in the oral immediate-release (IR) formulations (10 mg) in LMIC is 5.8 times higher than in HIC [33]. Moreover, in developing countries, it frequently happens that the cheapest formulation, such as the oral IR formulations, are sold at prices higher than transdermal or sustained-release formulations [34]. Furthermore, not all formulations of medicines are always available in these countries. Often, the ones most needed (oral morphine preparations, IR tablets) are missing. Therefore, many obstacles should be removed, not only in developing countries, to make opioid use more rational and accomplish patient-tailored pain management.

Consumption of the N02B group of drugs was constantly rising both in Serbia and Montenegro, as well as Serbian expenditures for these drugs. As far as the N02BB group is concerned (pyrazolones), only metamizole is present on the market of both countries. In this study, it was shown that neither consumption nor costs for metamizole changed significantly during the entire observed period. In our study published in 2018 [35], it was demonstrated that utilization of this drug was 3.31-fold higher in Serbia than in Croatia and that the expenditure of metamizole in the same period (from 2010 to 2015) was 5.29-fold higher in Serbia than in Croatia. Although metamizole use was gradually decreasing with a minimal value of 0.5 DDD/1000/day in 2015, it rose again and accounted for 1.91 DDD/1000/day in Serbia in 2019. Consumption in Montenegro was even higher, from 3.23 to 3.34 DDD/1000/day, while data from Croatian Medicine and Medical Devices Agency indicate that in the same period, like in our study, it was in the range from 0.41 to 0.92 DDD/1000/day [19]. In most European countries, metamizole has not been on the market for a relatively long time due to serious adverse effects, such as agranulocytosis, thrombocytopenia, aplastic and haemolytic anemia, etc. [36]. Therefore, although metamizole is widely used, its prescribing should be strictly based on the indications and appropriate duration of therapy according to its current Summary of product characteristics.

A consistently rising trend of both expenditures and consumption of paracetamol was noticed in the entire monitored period in both countries, only less prominently in Montenegro than in Serbia. Miljković et al. [35] showed that from

2010–2015 paracetamol was the most frequently prescribed analgesic in both Serbia and Croatia, and its consumption continued to increase during the whole period. This trend continued in both of these countries, with the average use from 4.7 to 5.0 DDD/1000/day, probably due to a very good risk/benefit ratio and affordable prices, which makes it the most commonly prescribed antipyretic and analgesic in children, not only in Serbia, Montenegro, and Croatia but also in Finland, as a representative country for comparison [19, 36]. Referring to the M01 group, the most prominent expenditures and consumption of all analgesics were recorded in both Serbia and Montenegro during the whole observed period. Consumption of the M01AB group of drugs (diclofenac, etodolac, ketorolac, etc.) was higher than that of the M01AE group (propionic acid derivatives) in Serbia during the observed period, except in 2018 when it was lower. In Montenegro, consumption of the M01AB group of drugs was prominently higher in comparison to the M01AE group during the whole five-year period. This trend has existed in Serbia for a long time, according to studies from 2005 onwards [35, 37]. Mijatović et al. [37] even showed that diclofenac accounted for 50% of NSAID consumption in Serbia from 2005–2008, followed in much smaller amounts by ibuprofen. A similar situation was noted in Croatia, while in Denmark, ibuprofen consumption was higher compared to diclofenac from 2005 onwards [38]. In 2018 and 2019, the consumption of the M01AB group of drugs was still high both in Serbia and Montenegro, compared to Croatia, while consumption and expenditures of ibuprofen and propionic acid derivatives were steadily rising in all three countries [19]. These findings can be explained by a higher awareness of the lower adverse effects rate of ibuprofen compared to the corresponding one in the M01AB group, especially diclofenac [38]. That was especially emphasized after the announcement of the European Medicines Agency in 2013 that diclofenac use was associated with increased cardiovascular risks similar to those of COX-2 inhibitors [39]. This regulatory action caused a significant reduction in overall diclofenac initiation, which varied by country since it was investigated in Scotland, England, Denmark, and the Netherlands [40]. Interestingly, there was no impact on discontinuation and variable impact on switching of diclofenac. One of the ways to overcome the present bad prescribing practices in Serbia and Montenegro is to target education and adherence to the principles of evidence-based medicine [41].

In Serbia and Montenegro, over the five years, the expenditures and consumption of coxibs were the lowest among other anti-inflammatory drugs. That can be explained by their high price and limited indications due to the profile of adverse drug reactions, necessitating a patient-tailored approach [42, 43].

The WHO List of Essential Medicines includes only three medicines under the “non-opioid and anti-inflammatory medicines”: paracetamol, acetylsalicylic acid, and ibuprofen. The most commonly used NSAIDs are aspirin (88 countries), ibuprofen (90 countries), diclofenac (74 countries), indomethacin (56 countries), and naproxen (27 countries). In the group of 15 countries, uniformly distributed into low, middle, and high-income countries, it appears that diclofenac and etoricoxib account for a third of the total consumption of NSAIDs. As far as these medicines are concerned, there was no significant difference in their use in countries with low, middle, and high incomes [44, 45].

We did not provide detailed insight into the consumption trend for all the specific types of NSAIDs since national studies related to analgesics are rare. Nevertheless, we believe our analysis of NSAIDs consumption and price contributes to the understanding of the main reasons for their continuous irrational use, especially in middle-developed countries such as Serbia and Montenegro.

Conclusion

In both Serbia and Montenegro, allocations for analgesics are substantial, with a rising trend noted for the five years of observation (2015–2019). However, only about 0.22% of the total expenditures for medicines in Serbia were spent on opioids. Moreover, the distribution and consumption in these two countries classified as developing ones are faced with more or less the same obstacles as other developing countries. Both have a low number of opioid medicine types, either as single active substance drugs or combined formulations. Despite necessary caution in prescribing practice, more should be done to remove administrative obstacles that make them barely accessible to patients. Moreover, education of both the general population and healthcare professionals could help dispel prejudices relating to the consumption of opioids in both countries.

Non-opioid analgesics in the M01A and N02B groups of drugs are most frequently used. That can be explained by numerous indications for their use and OTC status. More rational prescribing, taking into consideration drugs with lower incidence of adverse drug reactions, like ibuprofen, should be present in practice.

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Influence of DOACS and DOAC-REMOVE® on coagulation assays during thrombophilia testing in DOAC-treated patients

Uticaj DOAK i DOAC-REMOVE® na testove koagulacije u toku testiranja trombofilije kod bolesnika lečenih primenom DOAK

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Abstract

Background/Aim. Direct oral anticoagulants (DOACs) administration significantly interferes with coagulation assays. The aim of the study was to evaluate the effect of DOACs and DOAC-Remove® on coagulation assays during thrombophilia testing. **Methods.** The study was carried out from January 2019 to the end of June 2020. It included 30 DOAC-treated patients, 14 females and 16 males aged 23 to 63 (median age 47.6 years), tested for thrombophilia due to venous thromboembolism (VTE). Thrombophilia testing was performed using DOAC-Remove® tablets (activated charcoal). The results before and after DOAC-Remove® were compared. **Results.** Positive lupus anticoagulant (LA) results were observed in 20% apixaban, 100% dabigatran, and 70% rivaroxaban-treated patients, while in samples after DOAC-Remove®, the LA positivity was observed only in one from the apixaban group. Before DOAC-Remove®, the activated protein C (APC) resistance (APC-R) was measurable in 40% dabigatran and 80% rivaroxaban-treated patients, while, after using DOAC-Remove®, the APC-R was measurable in all cases. Comparing the results obtained from the samples before and after DOAC-Remove®, a difference was noted in relation to all dilute Russell's viper venom time (dRVVT) coagulation tests, except for the dRVVT ratio in the apixaban group. Clot-based methods for detecting the APC resistance were significantly affected by dabigatran and less by rivaroxaban. **Conclusion.** DOACs were practically inactivated after the addition of the DOAC-Remove®, which made it possible to perform analyses for the LA and APC-R testing freely and obtain relevant results.

Key words:
anticoagulants; blood coagulation tests; evaluation study; thrombophilia.

Apstrakt

Uvod/Cilj. Primena direktnih oralnih antikoagulansa (DOAK) značajno utiče na testove koagulacije. Cilj rada bio je da se proceni uticaj DOAK i DOAC-Remove® tableta (aktivni ugalj) na testove koagulacije tokom ispitivanja trombofilije. **Metode**. Istraživanjem, sprovedenim od januara 2019. do juna 2020. godine, obuhvaćeno je 30 bolesnika lečenih DOAK-om i testiranih na trombofiliju zbog venskog tromboembolzma (VTE). Bilo je 14 žena i 16 muškaraca, starosti od 23 do 63 godine (medijana 47,6 godina). Ispitivanje trombofilije izvršeno je upotrebom DOAC-Remove® tableta (aktivni ugalj). Upoređivani su rezultati pre i posle primene DOAC-Remove®. **Rezultati**. Pozitivni rezultati za lupus antikoagulantni (LA) test dobijeni su kod 20% bolesnika lečenih apiksabanom, kod 100% bolesnika lečenih dabigatranom i kod 70% lečenih rivaroksabanom, a u uzorcima posle DOAC-Remove® pozitivnost na LA dobijena je samo kod jednog bolesnika iz grupe lečnih apiksabanom. Pre primene DOAC-Remove®, rezistencija na aktivisani protein C (*activated protein C resistance* – APC-R) bila je merljiva kod 40% i 80% bolesnika lečenih dabigatranom, odnosno rivaroksabanom, dok je posle primene DOAC-Remove®, APC-R bila merljiva u svim slučajevima. Upoređivanjem rezultata dobijenih iz uzoraka pre i posle primene DOAC-Remove®, primećena je razlika u odnosu na sve testove vremena koagulacije izvršene razblaženim Russell-ovim zmijskim otrovom (*dilute Russell's viper venom time* – dRVVT), osim dRVVT u grupi bolesnika lečenih apiksabanom. Na koagulacionu metodu za otkrivanje APC-R značajno je uticao dabigatran, a manje rivaroksaban. **Zaključak**. Nakon primene DOAC-Remove® tableta, DOAK su praktično inaktivisani što je omogućilo izvođenje analiza za LA i APC-R i dobijanje relevantnih rezultata testova.

Ključne reči:
antikoagulansi; krv, testovi koagulacije; procena, istraživanja; trombofilija.

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Introduction

The new class of anticoagulants has been referred to as novel oral anticoagulants (NOACs). With regard to the mechanism of action, they are target-specific oral anticoagulant agents and are, as such, aimed at inhibiting a specific coagulation factor. Hence, dabigatran is a direct inhibitor of thrombin, while rivaroxaban, apixaban, and edoxaban are direct inhibitors of activated factor X (FXa). For the control of anticoagulation, the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) recommends the term direct oral anticoagulants (DOACs) [1]. DOACs are increasingly being used as an alternative to warfarin in the treatment of venous thromboembolism (VTE). However, the introduction of DOACs brought new challenges for all those involved in laboratory testing and assessing thrombophilia presence. These challenges include DOACs interference on coagulation (clot-based) assays, which can lead to false-positive or false-negative results (depending on drug concentration) [2–5]. Particularly problematic thrombophilia tests include clotting assays for proteins C and S, lupus anticoagulant (LA), and activated protein C resistance (APC-R) [6–9]. The most recent guidance from the Clinical and Laboratory Standards Institute (CLSI), published in 2014, gave a firm conclusion that LA testing was not recommended in DOAC-treated patients [10]. Concerning the results of the recently published study, all DOACs led to a prolongation in both dilute Russel's Viper Venom Time (dRVVT) based screen and confirm assays used for LA detection [11]. As a result of the extent prolongation of the dRVVT screen influenced by DOACs, mixing studies (ratio 1:1) recommended by the guidelines could not remove this interference, as it can do with vitamin K antagonist (VKA). Therefore, inhibition from the DOACs would still be present [12].

Despite this interference in coagulation assays, clinicians continue to request thrombophilia testing for DOAC-treated patients. The correct interpretation of thrombophilia testing results obtained in DOAC-treated patients is mandatory to prevent misclassification and subsequent clinical consequences [6].

The aim of the study was to evaluate the effect of DOACs and DOAC-Remove® tablets (activated charcoal) on coagulation assays during thrombophilia testing among our DOAC-treated patients.

Methods

Patients

The study included 30 DOAC-treated patients, 14 females and 16 males aged 23 to 63 years (median age 47.6 years), tested for thrombophilia due to VTE. The study was conducted from January 2019 to the end of June 2020. In all patients, thrombophilia testing was performed from a sample before and after the addition of DOAC-Remove® tablets. The results obtained before and after DOAC-Remove® tablets were compared. One of the DOAC-treated patients from the apixaban group was known to be LA-positive. The LA-positive test was diagnosed in this case upon low molecular weight heparin during the first VTE event.

Patient samples treated with DOAC-Remove®

In all subjects, whole blood was collected 2–12 hrs after DOACs intake into buffered sodium citrate tubes (containing 1/10 volume sodium citrate stock solution at 0.129 mmol/L; Vacutest, Kima) by sterile, atraumatic venipuncture. Two samples were taken for each patient. One was prepared by a standard method to obtain platelet-poor plasma for coagulation assays used in the thrombophilia testing and denoted as a sample before DOAC-Remove®. The second sample was prepared using DOAC-Remove® tablets. According to the instruction, 1 mL of previously prepared plasma sample was mixed gently for 5 min with a DOAC-Remove® tablet and then centrifuged for two min at 2,000 g. The supernatant obtained after centrifugation, denoted as a sample after DOAC-Remove®, was used for coagulation assays.

Thrombophilia testing included the following: screening tests, activated partial thromboplastin time (APTT), and prothrombin time (PT); determination of antithrombin (AT), protein C (PC), and protein S (PS) activity; presence of LA and APC-R. All assays were performed using Siemens Healthcare Diagnostics reagents on a BCS XP system (Siemens, Marburg, Germany) according to the manufacturer's instructions. Pathromptin SL and Thromborel S were used for APTT and PT testing, respectively. The activity of natural inhibitors was measured using Berichrom ATIII (anti-IIa based) and Innovance Antithrombin (anti-Xa based) for AT, Berichrom Protein C for PC, and Innovance Free Protein S Ag for PS. The presence of LA was evaluated using an integrated assay based on dilute Russel's viper venom time (dRVVT) tests that utilize dRVVT LA screen reagent (LA1) and dRVVT LA confirm reagent (LA2). Results for LA1, LA2, and LA ratio were expressed following CLSI recommendations published in 2014 [10] as normalized ratios, and a value of 1.2 was used as the cut-off value. APC-R was determined using the Russell Viper Venom Time (RVVT)-based functional clotting test (ProC Ac R assay). APC-R lower than 1.8 was considered pathological. Additionally, genotyping of FV Leiden and FII G20210A mutations was carried out in all study participants. The mutations were detected by polymerase chain reaction (PCR) followed by digestion with specific restriction enzymes (MnlI for FV Leiden and HindIII for FII G20210A, NEBiolabs). Normal and mutated alleles were distinguished by the size of the restriction fragments using electrophoresis on polyacrylamide gels. For one AT deficient patient revealed in routine thrombophilia testing, the PCR analysis for SERPINC 1 gene was performed afterward. In order to minimize the effect of acute thrombosis on thrombophilia testing, the blood samples were collected 6–8 weeks after the acute thrombosis. Concerning the defined indication for the thrombophilia testing, patients who developed their first thrombosis before the age of 50 were included in the study.

Institutional approval for the study was granted by the Local Research Ethics Committee (No 1101/2, from September 17, 2020) following internationally accepted ethical standards. Each patient signed the informed consent form.

Statistical analysis

The distribution of analyzed data was tested. Description of data was done using median and interquartile range (IQR). Differences between groups' data were evaluated using the Mann-Whitney *U* test and Fisher's exact test. A *p*-value less than 0.05 was considered statistically significant.

The Statistical Package for Social Sciences 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses.

Results

The clinical data of the study participants (age, sex, thrombotic manifestation, comorbid condition, and DOACs doses) are presented in Table 1.

Analysis of the results obtained among 30 DOAC-treated patients, denoted as samples before DOAC-Remove® and after DOAC-Remove®, are shown in Figures 1−3. Positive LA results were observed in 20% apixaban, 100% dabigatran, and 70% rivaroxaban-treated patients. In samples after DOAC-Remove®, the LA positivity was observed only in one from the apixaban group. Before DOAC-Remove®, the APC-R ratio was measurable in 40% of dabigatran and 80% of rivaroxaban-treated patients, while, after using DOAC-Remove®, the APC-R was measurable in all cases.

Table 1

Patient characteristics

Parameter	Apixaban	Rivaroxaban	Dabigatran
Current age (years), range (median)	43–63 (48.3)	23–63 (43.4)	40–61 (51.2)
Sex (m/f), n	6/4	7/3	3/7
Thrombosis localization, n			
DVT/PE	4**	7***	4*
PE	3*	2	4*
DVT	3*	1*	2*
Age of the first VTE (years), range (median)	41–49 (45.6)	19–50 (37.8)	40–49 (45.8)
Risk factors for the first VTE, n			
surgery	2	2	–
hormonal therapy	2	1	3
CA			1
Concomitant diseases, n			
CA	2	1	1
hypertension	1		
arrhythmia	1		
DOACs doses, mg	2 pts, 2 × 5 8 pts, 2 × 8	20	2 ×150

m – male; f – female; n – number of patients (pts); DVT – deep venous thrombosis; PE – pulmonary embolism; VTE – venous thromboembolism; * – patient with recurrent thrombosis, the number of stars refers to the number of pts in some groups; CA – breast cancer; DOACs – direct oral anticoagulants.

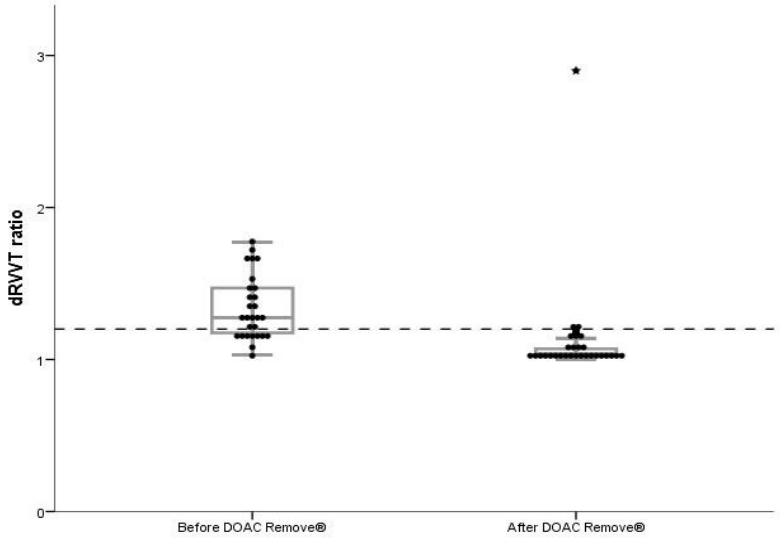

Fig. 1 – Results for dilute Russel's viper venom time (dRVVT) ratio of tested patients. Data are shown as individual dot plots with median and interquartile range (IQR) ratios for dRVVT ratio (y-axis). The samples are denoted as before and after DOAC-Remove® (x-axis).

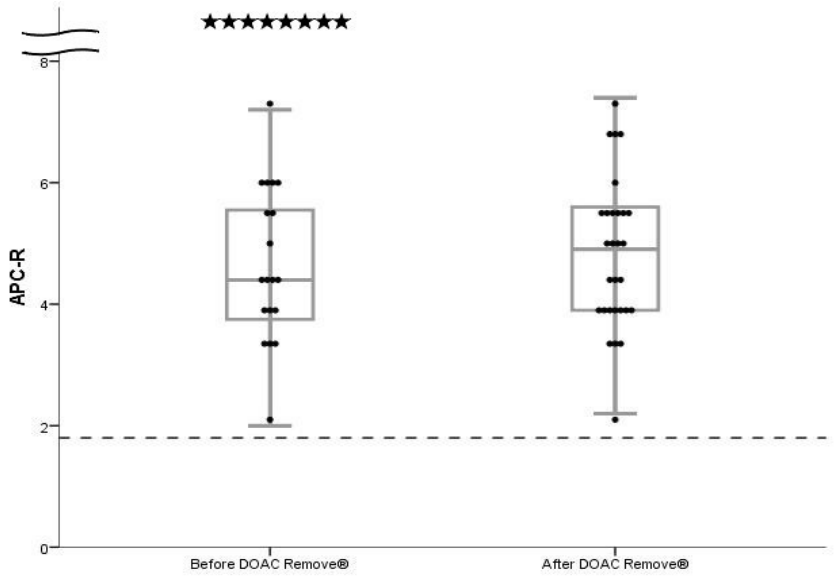

Fig. 2 – Results for activated protein C resistance (APC-R) of tested patients. Data are shown as individual dot plots with median and interquartile range (IQR) ratios for APC-R (y-axis). The samples are denoted as before and after DOAC-Remove® (x-axis). The dashed horizontal line presents a cut-off for APC-R (1.8) to define negative vs. positive test results. ★– immeasurable data.

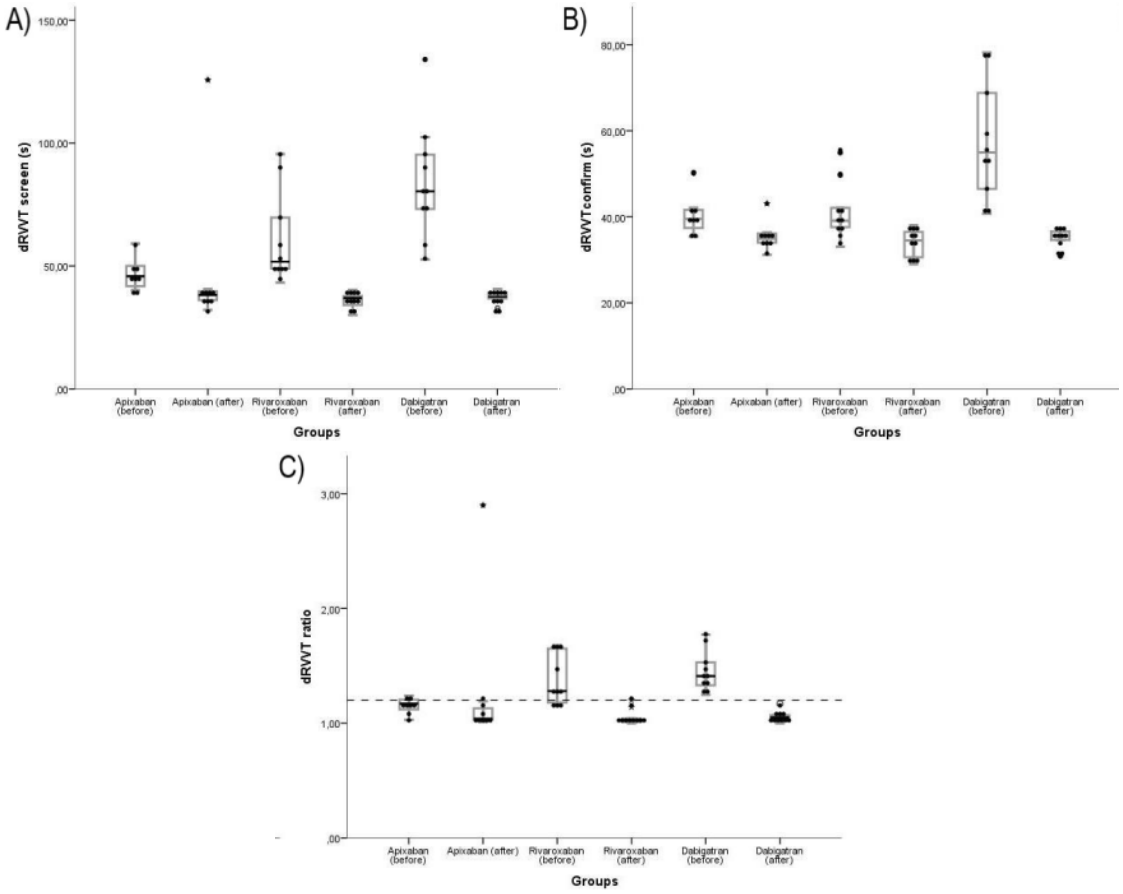

Fig. 3 – Results for: a) dilute Russel's viper venom time (dRVVT) screen; b) dRVVT confirm; c) dRVVT ratio of tested patients.

Data are shown as individual dot plots with median and interquartile range (IQR) ratios for dRVVT screen (A), dRVVT confirm (B), and dRVVT ratio (C) (y-axis). The patient groups are presented in relation to DOACs drugs (apixaban, rivaroxaban, dabigatran) before and after DOAC-Remove® (x-axis). The dashed horizontal line presents a cut-off for the dRVVT ratio (1.2) to define negative vs. positive test results.

Regarding the APC-R test results, PCR tests confirmed the obtained APC-R findings – the patients tested did not have the FV Leiden mutation.

A comparison of the coagulation assays results obtained before and after applying DOAC-Remove® showed a significant difference concerning the APTT only in the dabigatran group. A significant difference was observed in all DOAC-treated groups in relation to the dRVVT screen ($p = 0.012$, $p < 0.001$, $p < 0.001$) and dRVVT confirm ($p = 0.016$, $p = 0.005$, $p < 0.001$). In contrast, the difference in the dRVVT ratio was observed among rivaroxaban and dabigatran-treated patients ($p < 0.001$). In relation to the evaluation of the biological activity of natural inhibitors, there were no differences between samples before and after the addition of DOAC-Remove® tablets (Table 2).

One patient from the apixaban group, after treatment of the sample with DOAC-Remove®, was confirmed as an AT-deficient patient. Using an anti-IIa assay before applying DOAC-Remove®, AT activity of 73% was obtained, while after applying DOAC-Remove®, it was 61%. Using anti-factor Xa (anti-Xa) assay before applying DOAC-Remove®, AT activity of 106% was obtained, while after applying DOAC-Remove®, it was 54%. Repeated analyses of a new sample taken after one month showed similar results. Genetic analyses have confirmed the presence of AT deficiency type I.

Table 2

Coagulation assays obtained from plasma samples before and after the addition of DOAC-Remove® tablets

Coagulation assay	Apixaban*		Rivaroxaban**		Dabigatran	
	(median)	IQR	(median)	IQR	(median)	IQR
APTT (s)						
BDR	31.2	5.15	31.85	8.875	41.15	5.5
ADR	28.5	2.6	29.7	3.5	30.05	3.5
p	0.101		0.054		**< 0.001**	
PT (%)						
BDR	108	13	104.5	18.25	95.5	19.25
ADR	120	14	110.5	12.25	105	20.5
p	0.477		0.173		0.054	
Fibrinogen						
BDR	3.7	1.55	3.6	0.95	3.55	0.725
ADR	3.35	1.275	3.05	1.1	3.4	0.7
p	0.343		0.172		0.544	
dRVVT screen (s)						
BDR	45.8	9.125	51.75	26.575	80.4	27.7
ADR	38.2	4.75	36.95	4.833	37.5	3.275
p	**0.012**		**< 0.001**		**< 0.001**	
dRVVTconfirm (s)						
BDR	39.55	5	39.05	6.85	54.95	25.625
ADR	35	2.65	34.55	6.15	35.6	3.025
p	**0.016**		**0.005**		**< 0.001**	
dRVVT ratio						
BDR	1.17	0.108	1.28	0.478	1.41	0.255
ADR	**1.04	0.135	1.025	0.057	1.05	0.043
p	0.112		**< 0.001**		**< 0.001**	
AT (%)						
BDR	99	19	99.5	19.75	100	17.25
ADR	#93	30.5	93	11.75	99.5	19.75
p	0.132		0.161		0.762	
PC (%)						
BDR	124	47	91	29.5	118.5	33.75
ADR	120	44.5	92.5	31.25	118	32
p	0.536		0.97		0.97	
PS (%)						
BDR	94	16.5	84.5	36.75	109	35
ADR	93	13.5	82.5	37.75	108	33.5
p	0.965		0.91		0.97	
APC-R						
BDR	5.300	1.3	4.1	1.3	4	2.95
ADR	5.600	0.9	4.2	1.175	4.7	3
p	0.873		0.657		0.62	

***One patient with positive lupus anticoagulant (LA) and one with AT deficiency were diagnosed in the apixaban group;**PCR tests confirmed that one patient treated with rivaroxaban was a carrier of the *FII G20210A* mutation.**

BDR – before DOAC-Remove®; ADR – after DOAC-Remove®; APTT – activated partial thromboplastin time; APC-R – activated protein C resistance; AT – antithrombin; dRVVT – dilute Russel's viper venom time; PC – protein C; PS – protein S; PT – prothrombin time; IQR – interquartile range.

***p* – Mann-Whitney *U* test (significant values are bolded).**

Discussion

The results obtained from the samples denoted before DOAC-Remove® showed that the reliability of the tests used in the detection of LA and APC-R was uncertain. Most patients on dabigatran (100%), rivaroxaban (70%), and apixaban (20%) appeared to be LA positive, while in half of those on dabigatran and up to 20% on rivaroxaban, the APC-R test was not measurable. The results obtained from the samples before DOAC-Remove® confirmed that DOACs administration has a significant interference on coagulation assays, mostly to those based on the APTT principle. The analysis of the standard coagulation tests showed that APTT is most responsive to dabigatran. With regard to the specific assays, all DOACs interfere with most APTT-based assays that were used in the detection of LA, while clot-based methods for detecting the APC resistance are significantly affected by dabigatran and less by rivaroxaban.

Comparing the results obtained from the samples before and after DOAC-Remove®, a difference was noted in relation to all dRVVT tests, except for the dRVVT ratio in the apixaban group. Favaloro et al. [11] explained the difference between DOACs in LA testing, which was also confirmed in our study. They showed that rivaroxaban affected the screen more than the confirm, leading to higher RVVT ratios, while apixaban affected the confirm more than the screen, leading to lower RVVT ratios.

We have to note that in the apixaban group, one patient was diagnosed as an AT-deficient patient. In this particular case, in the sample before DOAC-Remove®, we found a discrepancy in the level of measured AT activity since that anti-Xa assay provided a normal AT level, while after DOAC-Remove®, both assays showed reduced AT activity. Ząbczyk et al. [13] showed that treatment with rivaroxaban and apixaban overestimates AT activity measured by FXa- but not FIIa-based assay in AT-deficient individuals. Due to the mechanism of inhibition (via FIIa or FXa), DOACs may interfere with AT activity tests based on the same principles. Therefore, the FIIa-based assay is preferred in patients treated with rivaroxaban or apixaban [14].

On the other hand, the analysis of PCR results confirmed the obtained findings of the APC-R test, obtained from samples denoted as after DOAC-Remove®. We have to emphasize that in relation to the uncertain findings of the APC-R test, we can always instruct the patient to perform PCR in order to confirm or exclude the FV Leiden mutation. However, in the detection of LA, we do not have such a possibility. It should be noted that the findings of the tests used in evaluating the LA presence are very significant, given its impact on the recurrent thrombosis risk, which is important for clinicians in assessing the anticoagulant therapy duration. Therefore, the use of *in vitro* drug adsorption with activated charcoal (DOAC-Remove® or DOAC-Stop®) is a useful tool for DOACs neutralization and subsequent LA diagnosis in patients treated with DOACs. That is supported by recently published studies showing that activated charcoal effectively reduced plasma DOACs concentrations leading to appropriate dRVVT results in up to 97% of VTE patients [15, 16]. The authors of the second-mentioned study suggest the use of DOAC-Remove® for every rivaroxaban sample and in positive apixaban and dabigatran samples [16]. Likewise, Favresse et al. [17] suggested the use of the DOAC-Stop® treatment in clinical practice to avoid potential misclassifications and clinical consequences.

One of the limitations of our study worth discussing is the missing data on DOACs concentration in plasma before and after DOACs removal. However, less than 1% of laboratories that perform routine coagulation screening tests, PT or APTT, could measure DOACs plasma concentrations [5]. It is known that DOACs have predictable pharmacokinetic and stable pharmacodynamics profiles, so their administration does not require coagulation monitoring or measurement of concentration and dose adjustment. Peak plasma levels for DOACs are reached 2–5 hrs after administration, and their half-life is between 7–14 hrs, while the minimum effect is observed directly before the next drug administration at least 18–24 hrs after the last dose [4, 6, 15]. Recent studies reported that the addition of activated charcoal tablet has been able to neutralize the highest likely clinical concentrations of apixaban, dabigatran, rivaroxaban, and edoxaban and provide measurable and appropriate results for routine screening and specialty coagulation tests in patients taking DOACs. At the same time, these products showed minimal influence on non-DOACs plasmas [16, 18]. Kopytek et al. [9] demonstrated that DOAC-Remove® reduced DOACs concentrations of apixaban (< 3–7 ng/mL, $p < 0.0001$) and dabigatran (all < 5 ng/mL, $p < 0.0001$), while rivaroxaban concentrations were abolished at almost 100% (all < 3 ng/mL, $p < 0.0001$). Moreover, Slavik et al. [19], using reference HPLC-MS/MS method, reported that half a tablet of DOAC-Stop® added to each 0.5 mL of plasma removed apixaban from 97.1%, dabigatran from 99.5%, and rivaroxaban from 97.9% of participants' plasmas, leaving in some samples residual concentrations of DOACs that do not affect coagulation. In another study by Favresse et al. [17], DOAC-Stop® treatment was efficacious in neutralizing DOACs in patient samples removing DOACs to residual levels lower than the limit of quantification of the corresponding DOACs assays.

Conclusion

DOACs were practically inactivated after the addition of the DOAC-Remove® tablets in the prepared plasma samples, which made it possible to perform analyses for the LA and APC-R testing freely and obtain relevant results. Since DOACs laboratory monitoring is not routinely performed in most clinical laboratories, using activated charcoal tablets to neutralize DOACs in samples collected immediately prior to re-dosing may be an optimal approach to enable the coagulation testing and the correct interpretation of results in patients taking DOACs.

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Conflict of interest statement

The authors declare no conflict of interest.

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Peripartum depression: current considerations on classification, biological importance, and therapeutic potential of neuroactive steroids

Peripartalna depresija: aktuelna razmatranja o klasifikaciji, biološkom značaju i terapijskom potencijalu neuroaktivnih steroida

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Key words:
depression; neurosteroids; peripartum period; postpartum period; therapeutics.

Ključne reči:
depresija; neurosteroidi; peripartalni period; puerperijum; lečenje.

Introduction

Various research results indicate that peripartum depression (PED) occurs in 13–19% of mothers, although there is no precise data [1, 2]. Extensive epidemiological studies of PED have not been conducted in Serbia, and the results generated on smaller samples (n = 195, n = 120) showed that postpartum depression occurred in 11% [3], i.e., 23.3% [4] of women, respectively. The estimate is that 50% of women with depression during pregnancy or postpartum are not diagnosed or treated [5]. If not diagnosed or treated, in addition to the negative effect of the disorder on the patients, PED can negatively impact the child's health and lead to dysfunctional dynamics in the whole family. In extreme cases, outcomes of untreated peripartum depression can be suicide or infanticide [6]. Suicidality is increased in women during the peripartum period [7, 8], and a recent study [8] demonstrated that the prevalence of suicidality in individuals with depression or anxiety occurring in the year preceding or following birth increased significantly from 2006 to 2017. Maternal suicide accounts for up to 20% of all postpartum deaths, representing one of the leading causes of maternal mortality in the perinatal period [6]. Untreated PED can compromise the attachment process in mother-infant dyad, cause the cessation of breastfeeding, lead to infant neglect and abuse, imposing a series of short- and long-term adverse effects on the child [9]. In this paper, we will put a spotlight on current dilemmas regarding diagnostic criteria for PED through classification systems, their applicability in an everyday clinical practice setting, as well as new evidence on the biological importance of neuroactive steroids and their potential for the development of new pharmacotherapeutic options.

Postpartum depression or peripartum depression?

In the International Classification of Diseases (ICD-10), the tenth revision currently in use in the Republic of Serbia, "Mental and behavioral disorders associated with the puerperium (starting within six weeks of birth)", represents a distinct category. Within this category, PED and postnatal depression could only be classified as a subcategory of mild mental and behavioral disorders, which is not elsewhere classified. It represents a spectrum of mood changes in the postpartum period (PP), including transient postpartum sadness, postpartum depression (POD), and postpartum or puerperal psychosis [10]. With this, ICD-10 excludes the possibility of diagnosing POD in patients whose symptomatology fulfills the criteria for moderate or severe depression with or without psychosis. In such patients, in everyday clinical practice, clinicians usually diagnose major depression without any determinant regarding pregnancy. National Guidelines for the Treatment of Depression issued by the Ministry of Health of the Republic of Serbia in 2013 recognize depression during pregnancy and POD as distinct clinical entities with recommendations for its treatment [11].

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Contrary to ICD-10, The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), offers more precise criteria for depression related to pregnancy and/or delivery, placing it as one of the specifiers of major depressive disorder – "with peripartum onset". This specifier can be added to the diagnosis of a major depressive episode regardless of its severity as long as symptoms that meet the diagnostic criteria for depression occur during pregnancy or four weeks after delivery [12]. DSM-5 has made a major step forward in the classification of peripartum mood disorders, given that a growing body of research evidence indicates that nearly 50% of peripartum depressive episodes have their onset during pregnancy [13, 14].

The Eleventh Revision of the International Classification of Diseases (ICD-11), which is effective as of January 1st, 2022, recognizes as a distinct diagnostic category "a syndrome associated with pregnancy or the puerperium (commencing within about six weeks after delivery) that involves significant mental and behavioral features, most commonly depressive symptoms" [15]. The name of this clinical-diagnostic entity – "postpartum" or "postdelivery" depression, has become rooted both among healthcare professionals and the general population. An increasing number of studies indicate that approximately 50% of peripartum depressive episodes have their onset during pregnancy, most often in the third trimester, and can deteriorate significantly in PP [13, 14, 16]. The international perinatal psychiatric consortium for POD conducted a large study examining the heterogeneity of a sample of 6,556 women with POD. Among the patients with the most severe symptomatology (n = 730), 67% had the onset of symptoms during pregnancy [14]. A study [17] conducted in 2019 on a sample of 2,466 participants from a large population cohort in Sweden examined the timing of the onset of PED and its association with various risk factors. This study showed that 60.6% had no depression, 8.5% had developed depression during pregnancy, 10.9% had depression with early postpartum onset, 5.4% with late postpartum onset, and 14.6% had chronic depression. There is also evidence that suggests that PED may occur within 12 months after delivery [17, 18]. Research data indicate that the peak time of the onset of PED symptoms occurs between the second and third postpartum month [19].

Insufficiently comprehensive diagnostic criteria for PED may have several implications for everyday clinical practice. Although progress is made through new revisions of the DSM-5 and ICD-11 classification systems, there appears to be a large discrepancy between narrow time windows proposed by diagnostic criteria and a growing body of evidence indicating a much broader time range for the manifestation of the disorder. That can lead to the failure of documenting this disorder with unreliable prevalence, especially in developing countries that do not have screening programs for peripartum depression. That may also contribute to underdiagnosing and, thus, undertreatment and insufficient research on this disorder. The lack of sufficiently comprehensive diagnostic categories can be an issue for both physicians and patients who develop mood disorder symptoms in the peripartum period. It may mislead the physician to a different therapeutic approach, and in administrative terms, it may limit patient access to certain specialized programs or new therapeutic options for peripartum depression. That can especially be a problem in countries with restricted healthcare budgets, where health insurance funds are forced to additionally restrict the criteria for reimbursement of certain medicines compared to official diagnostic criteria or the registered indication of the drug due to the high prices of innovative drugs.

The abovementioned imperfections of current classification systems must not be a barrier to the detection and treatment of peripartum depression. The term peripartum depression, accepted by DSM-5 classification, can be a strong signal for both clinicians and the general population that pregnant women can also develop mood disorder that requires treatment.

It is a widespread belief in the general population, and among clinicians as well, that pregnancy is a protective factor when it comes to mental health, but the situation is different. Research data indicate that many psychopathological manifestations occur during pregnancy for the first time [20]. Only 25% of women who develop symptoms of PED report symptoms to their physician. In addition, there is a tendency to minimize these symptoms both by the mothers themselves and healthcare workers, attributing them to physiological consequences of childbirth [21]. More intensive and comprehensive strategies for raising awareness and psychoeducation about this disorder are necessary. Furthermore, the diagnostic approach for PED should be more proactive and incorporate an extensive network of healthcare professionals who engage with pregnant women and women in the PP. There are currently no official programs or recommendations for screening for PED in the Republic of Serbia. Experiences from developed countries show that screening for PED led to an increase in diagnosis and treatment of the disorder only when specialized treatment was available. Moreover, some data show that screening for PED in the presence of an appropriate treatment program improves the treatment outcomes [22]. One study [23] showed that even when screening was mandatory, it did not lead to higher rates of treatment for POD – it led to high screening rates, but there were low rates of transition to treatment programs. One large cohort study [24] conducted in the USA on pregnant women (n = 97,678) examined the rates of screening and treatment before, during, and after the implementation of a universal perinatal screening program for depression in one obstetric clinic. Not only did this study show a significant increase in screening rates but also an increase in the number of women treated for depression. The authors emphasized that the main advantage of such a program was the well-established cooperation of obstetric clinics with healthcare professionals in the field of mental health. The authors also point out that, in this case, well-established and easily accessible ways of referring patients within the health system contributed to improved treatment outcomes.

Considering the great importance of diagnosing and treating PED, the development of a targeted national screening program for PED is imposing itself as a necessity. Furthermore, there is an unmet need for the development and implementation of well-established clinical pathways with well-defined steps – from suspected PED identified by screening to evaluation and evidence-based treatment in specialized mental health services. Given the number of different medical specialties that come into contact with women during the peripartum period, there is a big responsibility of each professional association to address the problem of PED in their guidelines and recommendations. However, great strides can be made at the individual level, including general practitioners, obstetricians, gynecologists, nurses and technicians in gynecological services, community nurses, pediatricians, as well as employees in mental health services. Their engagement and proactive approach in screening for PED and timely referral add great value to the overall management of the disorder.

Current considerations on the biological importance of neuroactive steroids in the development of peripartum depression

A plethora of biological and psychosocial research has demonstrated that the etiology of PED is multifactorial, with complex yet not completely understood underlying pathophysiology that leads to a myriad of different phenotypes [14, 25]. Here, we scrutinize recent research on the role of neuroactive steroids in the development of the disorder and the implications they have on the development of new pharmacological options for PED. We particularly focused on recent research on the interplay of perinatal neuroactive steroids perturbations with the hypothalamic-pituitary-adrenal (HPA) axis, gamma-aminobutyric acid (GABA)-mediated neurotransmission, and neuroinflammation.

Studies have demonstrated that basal cortisol concentrations increase during pregnancy, reaching their peak in the last weeks. In days and weeks after delivery, there is a decrease in corticotropin-releasing hormone (CRH) and cortisol concentrations, and that decrease was related to the development of depression with postpartum onset. It has also been shown that lower cortisol concentrations registered in women with POD are maintained for up to 12 months after delivery [26]. The inability to suppress the induced activation of the HPA axis during pregnancy and the PP is considered to play a dominant role in the pathogenesis of PED [27]. Some nonclinical studies [28, 29] have shown a critical role for K+/Cl- co-transporters – (KCC2) in GABA-mediated regulation of CRH neurons in the paraventricular nucleus of the hypothalamus and subsequent regulation of stress-induced HPA axis activation. These studies have demonstrated the role of chloride homeostasis in regulating the physiological stress response. Data from nonclinical studies indicate that HPA axis suppression during pregnancy and the PP is mediated by normal maintenance of KCC2 expression in the paraventricular nucleus of the hypothalamus. On the contrary, selective loss of KCC2 in neurons with CRH results in the inability to suppress the HPA axis during pregnancy and PP, which was related to anxiety and depression-like behavior in mice. A recent study [29] directly linking the HPA axis and PED in mice showed that chemogenetic "silencing" of CRH neurons in the periventricular nucleus of the hypothalamus might improve abnormal postpartum behavior observed in mice with selective KCC2 loss. These data suggest that regulation of KCC2 activity is a potential target for developing new pharmacological options for peripartum depression.

As indicated above, there is a functional connection between GABA transmission and HPA axis dysfunction, and studies have shown that disrupted GABA signaling results in the absence of HPA axis suppression in PED [29–31]. During pregnancy and in the PP, there are significant changes in the concentrations of neuroactive steroids – metabolites of steroid hormones that manifest their effects in the central nervous system [32]. It has been shown in animal models that the neuroactive metabolite of progesterone, allopregnanolone, exerts anxiolytic and antidepressant effects that are considered to be mediated, at least partially, by its ability to potentiate GABA receptors by positive allosteric activity [33].

The GABA system adapts to changes in the concentrations of neuroactive steroids during pregnancy [34, 35]. Allopregnanolone concentrations increase during pregnancy and reach their maximum during the third trimester [36–38], followed by a steep drop in concentration after delivery [39]. As neuroactive steroid concentrations increase during pregnancy, GABA type A ($GABA_A$) receptors downregulate. Under physiological conditions, postpartum concentrations of neuroactive steroids lead to the re-expression of $GABA_A$ receptors. It is hypothesized that in PED, the level of $GABA_A$ receptor expression does not return to the previous one, leading to impaired neuroplasticity of $GABA_A$ receptors and the absence of a homeostatic mechanism that maintains the ideal level of $GABA_A$ transmission in response to fluctuating neurosteroid concentrations [34, 35]. These findings on the role of neuroactive steroids and $GABA_A$ receptor regulation in the pathophysiology of POD represent an important basis for developing innovative therapeutic options for PED.

Another important mechanism that shed light on the area of the pathophysiology of PED in this research is the interrelationship of neuroactive steroids, neuroinflammation, and tryptophan catabolism. In physiological conditions, pregnancy is characterized by a balance between inflammatory and anti-inflammatory mechanisms [40]. These mechanisms progress towards the preponderance of a "proinflammatory state" while approaching end-of-term pregnancy [40] leading to increased immune regulatory processes, partly via immune mechanisms and partially via increased HPA-axis activity and progesterone levels [41]. As discussed, these mechanisms could be disrupted after delivery leading to increased serum levels of proinflammatory cytokines. Although the overall data from studies are not convincing [40], some of them significantly

and positively associated increased levels of interleukin (IL)-6 [42–44], IL-1β [42], tumor necrosis factor (TNF)-α [43], and IL-8 [44] with depressive symptoms in postpartum women. It is implicated that increased levels of proinflammatory cytokines activate and shift tryptophan metabolism towards its degradation via the kynurenine pathway, which limits serotonin production and leads to an imbalance of neurotoxic and neuroprotective tryptophan catabolites that compromise glutamatergic neurotransmission contributing to depressive and anxiety symptoms in women with POD [44]. Furthermore, besides neuroinflammation, there is some evidence that both progesterone and estrogen have their role in regulating tryptophan degradation by suppressing two important enzymes of the kynurenic pathway – indoleamine 2,3 dioxygenase (IDO) [45] and tryptophan decarboxylase (TDO) [46]. These mechanisms could be important in PED since the placenta exhibits high expression and activity of these kynurenine pathway enzymes [47] and knowing that there is a dramatic drop of estrogen and progesterone after childbirth that could potentially contribute to increased tryptophan catabolism with already described effects.

The pharmacotherapeutic potential of neuroactive steroids in peripartum depression

Currently, pharmacotherapeutic interventions in PED, as well as in other disorders related to women's hormonal transition phases, mainly consist of off-label use of monoaminergic antidepressants approved for the treatment of major depressive disorder [11, 48–52]. To date, no drug in the Republic of Serbia nor the European Union has received regulatory approval for PED. This situation created a huge unmet need for developing specific, effective therapies for treating peripartum depression. The development of new therapeutics for PED was primarily determined by progress in elucidating the complex pathophysiology of the disorder, but also the delicacy of conducting clinical trials in this population of patients.

The already mentioned role of allopregnanolone in the positive allosteric modulation of GABA receptors was the scientific basis for the development of brexanolone, the first specific therapy approved by the United States Food and Drug Administration (FDA) for the treatment of POD in adults in 2019 [53]. Due to the poor oral bioavailability of allopregnanolone and its rapid metabolism, an analog of allopregnanolone – brexanolone, whose intravenous application achieves stable serum concentrations, has been developed [54]. The exact mechanism of action of brexanolone is not completely clear. However, it is considered that brexanolone offers women with POD comparable concentrations of allopregnanolone with concentrations of endogenous allopregnanolone in the third trimester of pregnancy, providing additional time for physiological adaptation to abruptly reduced concentrations of endogenous allopregnanolone. In addition, since it acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors, it thus exhibits acute anxiolytic and antidepressive effects [55]. Brexanolone was registered based on efficacy and safety studies in one open-label proof of concept study, one double-blind placebo-controlled phase 2 study, and two double-blind, placebo-controlled phase 3 studies [54–56].

Both phase 2 [54] and two-phase 3 studies [56] enrolled patients whose onset of PED was during the third trimester of pregnancy or within four weeks of delivery. Enrolled patients had severe depressive episodes at the start of treatment in phase 2 and the first phase 3 study and moderately severe depression in the second phase 3 study. In all three studies, brexanolone was administered as a continuous intravenous infusion for 60 hrs, with dose titration.

In phase 2 study [54] (n = 21), brexanolone showed a significant effect compared to placebo in the reduction in Hamilton Depression Rating Scale–Depression (HAM-D) overall score at 60 hrs post-infusion. In phase 3 studies with a larger number of patients (n = 122, n = 104), considerably smaller yet statistically significant effects were demonstrated [56]. In phase 2 study and the first phase 3 study, the achieved effects of the drug on the reduction of HAM-D scores were sustained until day 30 (longest follow-up). In the second phase 3 study, there was no difference between brexanolone and placebo on day 30 on the reduction of HAM-D scores [54, 56]. A pooled safety analysis from placebo-controlled studies on 140 patients with POD who received brexanolone showed that adverse events (AEs) leading to dose reduction or discontinuation of therapy were related to excessive sedation (loss of consciousness, syncope, somnolence, dizziness, fatigue), infusion site reactions, changes in blood pressure, or infusion pump dysfunction. Due to serious AEs in the form of sedation, somnolence, and loss or altered state of consciousness, FDA approved brexanolone with significant restrictions. The drug may be administered only in specialized and certified institutions with trained health care staff with the obligation of risk assessment and risk mitigation strategy implementation and constant patient monitoring for hypoxia using a pulse oximeter with an alarm for all 60 hrs of continuous drug administration. The drug has not been tested on pregnant women and should not be used during pregnancy [57].

Presented data suggest that treating patients with PED with this drug would be possible only in carefully selected cases, with extreme caution. Given that the longest follow-up in brexanolone studies was only 30 days, it is still unclear what the long-term treatment outcomes are. Gathering these data outside controlled clinical studies in everyday clinical practice would be very valuable for making more informed therapeutic decisions. Another pitfall that could hinder the widespread use of brexanolone in everyday clinical practice is the need for its administration in the inpatient setting, even if the presenting patient symptomatology does not require hospitalization. That would lead to early mother-infant separation with a tendency to its short and long-term implications. The manufacturer of brexanolone is currently developing another compound for the treatment of POD – zuranolone, with recently completed phase 3 clinical trial [58], which we appraise below. Zuranolone is an oral formulation with a similar pharmacological profile as brexanolone,

exerting its action as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors. While brexanolone is identical to allopregnanolone, zuranolone has a neuroactive steroid base that has been chemically modified to increase its oral bioavailability. In this phase 3, double-blind, placebo-controlled study (n = 153), zuranolone was administered orally once a day in patients with severe POD. Zuranolone improved symptoms of depression compared to placebo on day three, and the improvement was sustained until the forty-fifth day (longest follow-up), even with dosing cessation after 2 weeks. In this trial, zuranolone led to rapid and sustained improvements in anxiety as well as improved global and maternal functioning compared with the placebo, despite the relatively high placebo response observed in this trial. Zuranolone was well-tolerated. The same percentage of treatment-emergent AEs was recorded in both groups, with three patients in the zuranolone group (sedation, n = 1; confusional state, n = 1; migraine, n = 1). In the zuranolone group, one patient discontinued because of an AE (intermittent sedation). The most common AEs in the zuranolone group were somnolence, headache, dizziness, upper respiratory tract infection, diarrhea, sedation, and nausea. Unlike brexanolone, zuranolone in this outpatient randomized clinical trial did not lead to any notable or clinically significant changes in vital signals or electrocardiograms. No evidence for increased suicidal ideation or suicidal behavior was observed compared with the baseline, measured by the Columbia-Suicide Severity Rating Scale. These data from the zuranolone study are very encouraging considering the oral route of administration in the outpatient setting, fast response, and rapid achievement of remission with a favorable safety profile. Furthermore, in addition to improvement of clinician-rated measures of depression, anxiety, and global functioning, an important differentiator for zuranolone is the evidence of improved maternal functioning at day 45 recorded as a patient-reported outcome measure. What we see as the downside of presented data is the lack of long-term treatment outcomes as well as the safety of the drug in breastfeeding mothers. Moreover, what may narrow the indication field of use for this drug is the fact that zuranolone is currently investigated only in patients with severe POD, while patients who require treatment mostly have mild and moderately severe POD. With all the available data for these two innovative neuroactive steroid $GABA_A$ receptor-positive allosteric modulators, it seems more likely that zuranolone, if approved by health authorities, has greater potential than brexanolone to become the new standard of care for patients with severe PED with broader adoption in clinical practice.

Conclusion

The research data show that PED may occur during pregnancy, most often during the third trimester and within one year after delivery. Complex interactions of neuroactive steroids, neuroinflammation, and neurotransmitters represent an area of intensive research in an attempt to elucidate the biological basis of PED but also an area that stimulates the development of new pharmacotherapeutic options. Data from late-stage clinical trials of brexanolone and zuranolone are promising but more evidence from everyday clinical practice on long-term safety, efficacy, and functional outcomes would better inform new therapeutic algorithms for peripartum depression. There is still an urgency to do research both on underlying pathophysiology and the development of new medications for PED since this area is lagging far behind other affective and other mental health disorders.

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CASE REPORTS

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Lymphoma of the uterine cervix – a rare clinical presentation

Limfom grlića materice – retka klinička prezentacija

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Abstract

Introduction. Lymphomas are malignant diseases of the lymphocyte lineage. There are two basic types of lymphoma: Hodgkin's lymphoma (HL), whose main characteristic is the presence of Reed-Sternberg cells, and non-Hodgkin's lymphoma (NHL), which presents a heterogeneous group of diseases, and depending on the growth rate and the course of the disease, they can be indolent (slow-growing) and aggressive (fast-growing). Follicular lymphoma (FL) is the most common indolent form of NHL, while diffuse large B-cell lymphoma (DLBCL) is the most common aggressive form. **Case report.** The study presents a case of NHL, DLBCL, localized in the cervix, histopathologically diagnosed in a 35-year-old woman who, after a cervical biopsy, was histopathologically diagnosed with mild dysplasia (CIN1/L-SIL) of the cervical epithelium and, after that, an infection with human papillomavirus (HPV) subtypes 16 and 31 was proven. The diagnosis of DLBCL was histopathologically confirmed on a conical section of the vaginal portion of the uterus, after which the disease was treated with eight cycles of chemotherapy according to the RCHOP protocol. **Conclusion.** The coexistence of CIN1/L-SIL and NHL is random. However, this fact may cause the concomitant cervical lymphoma to be overlooked since the lymphoma is usually subepithelial if biopsies are not taken adequately and if HPV serotyping of the biopsy sample is not performed.

Key words:
biopsy; lymphoma, non-hodgkin; papillomaviridae; serotyping; uterine cervical dysplasia.

Apstrakt

Uvod. Limfomi su maligne bolesti limfocitne loze. Postoje dva osnovna tipa limfoma: Hodgkin-ov limfom (HL), čija je osnovna karakteristika prisustvo Reed-Sternberg-ovih ćelija, i non-Hodgkin-ov limfom (NHL), koji predstavlja heterogenu grupu bolesti, a u zavisnosti od brzine rasta i toka bolesti mogu biti indolentni (sporastući tok) i agresivni (brzorastući tok). Folikularni limfom (FL) najčešći je indolentni oblik NHL, dok je difuzni krupnoćelijski B-limfom (*diffuse large b-cell lymphoma*, DLBCL) najčešći agresivni oblik. **Prikaz bolesnika.** U radu je prikazana bolesnica stara 35 godina sa NHL, DLBCL, lokalizovanim u grliću materice, kojoj je nakon biopsije grlića materice, patohistološki dijagnostikovana displazija lakog stepena (CIN1/L-SIL) pokrovnog epitela grlića materice, a zatim dokazana infekcija humanim papiloma virusom (HPV), podtipovima 16 i 31. Dijagnoza DLBCL je patohistološki potvrđena na koničnom isečku vaginalne porcije uterusa, nakon čega je bolesnica lečena sa osam ciklusa hemioterapije po protokolu RCHOP. **Zaključak.** Koegzistencija CIN1/L-SIL i NHL je slučajna. Usled toga, može se prevideti prateći cervikalni limfom, koji je uobičajeno lokalizovan subepitelno, ukoliko se biopsije ne uzimaju na adekvatan način i ukoliko se ne uradi HPV serotipizacija uzorka biopsije.

Ključne reči:
biopsija; limfom, nehodžkinov; papillomaviridae; serotipizacija; grlić materice, displazija.

Introduction

Primary non-Hodgkin's lymphoma (NHL) of the cervix is extremely rare. Only 0.5% of extranodal lymphomas in women originate from the female genital tract. To date, more than 130 cases of primary NHL of the cervix have been reported [1].

The most common histological subtype is diffuse large B-cell lymphoma (DLBCL). The symptomatology is diverse.

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Fever, night sweats, and weight loss are not usually the characteristics of primary cervical lymphoma. Gynecological symptoms may occur, including pelvic pain, postcoital bleeding, postmenopausal bleeding, and dyspareunia. The differential diagnosis of primary cervical lymphomas includes benign inflammatory and malignant diseases such as cervical cancers, sarcomas, and lymphoma-like lesions.

The diagnostic procedure of choice for the final diagnosis is a deep cervical biopsy that detects histopathological characteristics and immunophenotype. Because they are subepithelial, Papanicolaou (PAPA) swabs play a very insignificant role in the diagnosis of cervical lymphoma. Ann Arbor stage, disease extent, number of affected extranodal organs, performance status, and serum lactate dehydrogenase (LDH) values present significant prognostic features [2].

Case report

A 35-year-old female patient was diagnosed with cervical intraepithelial neoplasia (CIN)1 / low grade squamous intraepithelial lesion (L-SIL) during a routine gynecological examination, which included a PAPA test. After that, curettage of the cervical canal and cervical biopsy were performed, and the diagnosis of CIN1/L-SIL was confirmed by pathohistological findings. The presence of the human papillomavirus (HPV) infection was proven after that (subtypes 16 and 31). After adequate preoperative preparation, a large loop excision transformation zone (LLETZ) conization was carried out. The diagnosis of CIN1/L-SIL and DLBCL was set by histopathological processing of the obtained cone-shaped portion of the cervix. The histopathological finding showed that in the obtained tissue samples of the vaginal portion of the uterus, there was a pronounced mixed inflammatory infiltration, composed of lymphocytes, plasma cells, and polymorphonuclear leukocytes, with a larger number of reactively altered follicles. Interfollicular lymphoid tumor tissue of diffuse growth type was present, which consisted of medium- and large-sized cells, rare slightly basophilic cytoplasm, a large oval, easily cut vesicular nucleus with 1-3 nuclei, which morphologically corresponded to immunoblasts and centroblasts. The mitotic and apoptotic indices were high. Necrosis was absent. There were no tumor cells at the edges of the cone resection. Immunohistochemically, tumor cells demonstrated diffuse immunoreactivity to LCA+, CD20+, bcl-6+, Vimentin+, Pax5+, with a high proliferative index of Ki-67 of about 50% (Figure 1). The patient was diagnosed with primary cervical diffuse B-large cell non-Hodgkin's lymphoma, centroblastic, germinal center B-cell-like (GCB) subtype.

After the diagnosis, the patient underwent multidetector computerized tomography (MDCT) (Figure 2) of the chest, abdomen, and small pelvis, as well as a postoperative gynecological examination, during which no secondary deposits were diagnosed. The patient was referred to the Hematology Clinic for further treatment.

A bone marrow biopsy was performed with a normal finding, without elements of the lymphoproliferative disease. The Hematology Council suggested that the patient be treated with eight cycles of chemotherapy (HT) according to the RCHOP protocol [rituximab, cyclophosphamide, hydroxydaunorubicin hydrochloride (doxorubicin hydrochloride), vincristine (older name oncovin), prednisone], with an assessment of the response after the second cycle. The patient was monitored by a hematologist after each cycle of HT, which she tolerated well due to the administration of antiemetics and gastroprotective therapy.

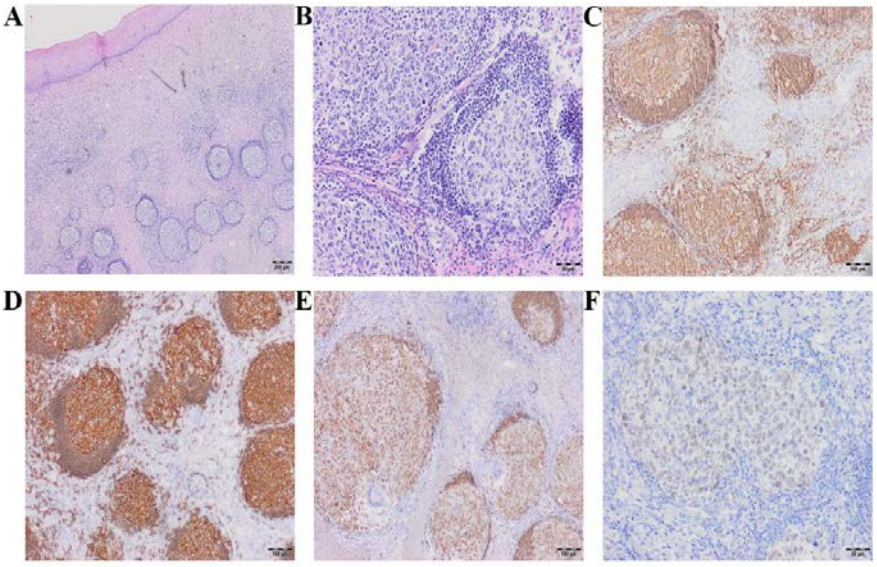

Fig. 1 – Histological and immunohistochemical features of *cervix uteri* lymphoma. Hematoxylin and eosin staining: A) ×400; B) ×200 original magnification; Immunohistochemical staining: C) diffuse positivity of the LCA (×100 original magnification); D) CD20 (×100 original magnification); E) PAX5 (×100 original magnification); F) Bcl 6 (×200 original magnification).

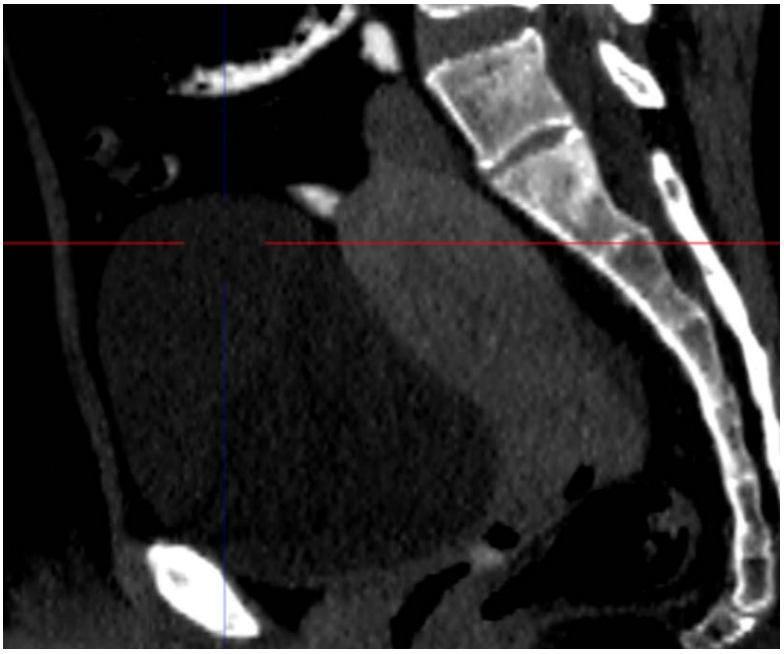

Fig. 2 – Computed tomography (CT) of the pelvis. Sagittal sections, prolapse of soft tissue mass from cervix *uteri* to the proximal third of the vagina. There are no CT signs of the infiltration of surrounding adipose tissue and organs.

After starting the hematological therapy, the patient has been regularly examined by a gynecologist every 4 months. Initially, there have been signs of increased vaginal secretion under the speculum. *Portio vaginalis uteri* (PVU) was voluminous, about 4–5 cm in diameter. At the control gynecological examinations, 4 months after the administration of HT, it was noticed that the volume of the cervix decreased (cervix 3 cm long, up to 4 cm wide). The findings of gynecological examinations, colposcopy, and PAPA tests were in order, as well as the exploratory curettage of the endocervical canal and endometrium – (pathohistological finding: secretory altered endometrium).

Discussion

Malignant lymphomas originate from the cells of the immune system - lymphocytes. There are more than 30 types of lymphoma, and each represents a disease with different biological behavior. This biological heterogeneity leads to significant differences among lymphomas in terms of epidemiology, pathohistological characteristics, and clinical presentation [3]. The NHL is ranked as the 5th to 9th most common malignancy in most countries around the world [4].

Primary lymphomas of the female genital tract are extremely rare, representing 0.2–1.1% of extranodal NHL and less than 0.5% of total gynecological malignancies [5].

Histological classification of all species is based on World Health Organization (WHO) standards. The most common histological type of lymphoma of the female genital tract, DLBCL, has several recognizable histopathological characteristics, with a poor prognosis and a preference for central nervous system dissemination [6].

According to the Globocan database, available on the Internet in 2020, uterine cervix neoplasms are in fourth place in terms of frequency. Precursors of cervical neoplasms originate from any squamous, glandular cell lineage (0.2%) or lymphocytes (0.1%) [7].

Clinical features, cervical biopsy, especially conization, and detailed immunohistochemical analyses are crucial for diagnosing cervical lymphoma [8].

In the United States, the incidence of NHL has increased significantly in recent decades and accounts for approximately 4% of all malignancies today [9]. Lymphomas are extremely rare tumors of the uterine cervix. DLBCL is the most common tumor of all lymphomas that can be localized at that site [10].

Immunodeficiency or autoimmunity is crucial for an increased risk of developing DLBCL. In addition, DLBCL can be caused by a large number of etiological factors such as infectious agents, pesticides, organic solvents, long-term use of hair dye, ultraviolet radiation, some drugs, genetics, and diet. Therefore, persistent antigen stimulation plays an important role in the development of lymphomas, especially extranodal lymphomas.

The WHO defines DLBCL as a neoplasm of large B lymphoid cells of a diffuse mode of growth whose nucleus size is close to or larger than macrophage nuclei or more than twice the size of lymphocytes. This tumor has clear histogenesis and originates from lymphocytes, cells of the immune system. Cells of DLBCL origin are considered to be centroblasts and immunoblasts. Centroblasts are located in the germinal center of the lymph follicle. Immunoblasts are located in the paracortex and are also called activated B cells.

Macroscopically, the cross-sectional area of the affected lymph node or extranodal organ is homogeneous, grayish-white in color, and bacon-like in appearance. Depending on the presence of areas of bleeding or necrosis, the cross-section area may be pink or yellowish, with a softened consistency. The histological picture is not uniform in all DLBCL subtypes.

These tumors have aroused interest due to their unique subepithelial localization and diverse clinical presentation. DLBCL is a disease of all ages but most commonly occurs in middle and old age. In most cases, DLBCL begins in the lymph nodes. Approximately 71% of patients have extranodal involvement during primary nodal disease. The most common sites of the occurrence of extranodal disease are the stomach and ileocecal region, but any part of the body may be the primary site of the disease, for instance, skin, bone, testis, spleen, Waldeyer's ring, salivary glands, thyroid, liver, kidney, adrenal gland, and uterus cervix. The disease can be localized or disseminated.

Initially and during disease monitoring, all patients should have a complete blood count, biochemical analysis including lactate dehydrogenase (LDH), and a bone marrow biopsy. Chest radiography and computed tomography (CT) scans of the chest, abdomen, and pelvis are also required. Functional scans, such as positron emission tomogrpahy (PET) scans, can enhance internships, especially by detecting occult diseases in the abdomen or spleen.

DLBCLs are aggressive but potentially curable with combined chemotherapy. DLBCL is a systemic disease; therefore, the therapy is systemic. Complete remission is nowadays achieved in 75–80% of patients, with long survival in about 50% of patients. The CHOP protocol has been the backbone of the therapy for decades.

Morphological, biological, and clinical studies have classified DLBCL into morphological variants, immunophenotypic, and molecular subtypes. There are also a large number of cases that may be biologically heterogeneous but for which there are no clear or accepted criteria for classification. They are classified as DLBCL, not otherwise specified (NOS), i.e., without a more precise specification, and include all cases that do not belong to specific subtypes.

Based on cytological characteristics, existing morphological subtypes of DLBCL are the following: centroblast, immunoblast, anaplastic, and rare morphological variants. DLBCL has two molecular subgroups: GCB and activated B-cell-like (ABC). Based on the immunophenotype, they are classified into the following: CD5-positive DLBCL, GCB, and non-GCB.

The Ann Arbor classification system is used to determine the stage: stage I – involvement of one region of lymph nodes or one extranodal organ; stage II – involvement of two or more lymph node regions on the same side of the diaphragm or localized involvement of the extranodal organ and one or more lymph node regions on the same side of the diaphragm; stage III – lymph node involvement on both sides of the diaphragm, with or without localized involvement of extranodal tissue or organ, spleen, or both; stage IV- diffuse or disseminated involvement of one or more distant organs, with or without lymph node involvement. Body temperature over 38 °C, night sweats, and/or weight loss over 10% for six months are called systemic symptoms and are denoted by the suffix B; asymptomatic patients are denoted by the suffix A.

Persistent HPV infection can cause carcinogenesis by hyperactivation of the immune system. One national cohort study examined the correlation between HPV infection and Hodgkin's and non-Hodgkin's lymphoma risk. All women who underwent conization between 1978 and 2011 were identified. In 87,435 women who underwent conization, an increased incidence of Hodgkin's and only a slight increase for non-Hodgkin's lymphoma was observed, but a correlation of HPV infections with an increased risk of lymphoma was proven [11].

The American Cancer Society (ACS) recommends that people over 25 should begin screening for cervical cancer and undergo primary testing for HPV every 5 years up to 65 years of age. Persistent high risk HPV (hrHPV) infection, primarily HPV types 16 and 18, are the cause of almost all cervical cancers. Although HPV infections are common in healthy adults, only a small proportion of infections survive and progress to precancerous cells in the cervix. This progression to a precancerous condition lasts for years. Thus, although HPV infections and CIN are common, they rarely lead to cervical cancer. Understanding HPV infection as the main causative factor for cervical neoplasia provided the basis for the introduction of HPV testing, which is a reliable predictor and risk element for precancerous and cervical neoplasia [12].

Conclusion

Lymphomas occur at all ages. Cervical lymphomas are extremely rare. The coexistence of CIN1/L-SIL and NHL may be completely random, and concomitant cervical lymphoma may be overlooked if biopsies are not performed adequately. Determining the presence of HPV infection is a significant predictive factor in the development of cervical lymphoma. Deep cervical biopsy, conization, and detailed immunohistochemical analyses are crucial for diagnosing cervical lymphoma. The differential diagnosis of primary cervical lymphomas includes benign inflammatory and malignant diseases such as cervical cancers, sarcomas, and lymphoma-like lesions. Since lymphomas are subepithelial, in the absence of ulceration, the PAPA swab plays a very insignificant role in the diagnosis of cervical lymphoma, but determining the presence of HPV infection is very significant. The key to diagnosis is histopathological analysis with the determination of immunophenotypic characteristics.

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Electrical shock-induced atrial fibrillation

Fibrilacija pretkomora uzrokovana strujnim udarom

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Abstract

Introduction. An electrical injury can cause various cardiac arrhythmias, such as asystole, ventricular fibrillation, sinus tachycardia, and heart blocks. However, it rarely causes atrial fibrillation (AF). **Case report.** The 47-year-old patient was admitted to the Emergency Department after receiving an electric shock (< 600 V). He subsequently lost consciousness, fell, and sustained back and head injuries. During the examination, the heart rate was irregular but with no heart murmurs. There was an entry wound on the front of the left thigh and an exit wound on the front of the neck. An electrocardiogram showed newly appearing AF. The laboratory tests showed no pathological deviation, and focus cardiac ultrasound showed that contractile force was preserved with no wall-motion abnormalities and normal left atrium dimensions. The patient was administered low-molecular-weight heparin subcutaneously and propafenone (600 mg) orally. At follow-up after 24 hrs, the electrocardiogram showed normal sinus rhythm. **Conclusion.** We reported a rare case of an electricity shock-induced AF, which was converted to sinus rhythm with the help of drug therapy. Although most cases of electricity shock-induced AF represent benign conditions that are self-limited, cardiac monitoring as a routine measure should be considered.

Key words:
anti-arrhythmia agents; atrial fibrillation; drug therapy; electric injuries; electrocardiography.

Apstrakt

Uvod. Strujni udar može dovesti do različitih poremećaja srčanog ritma poput asistolije, ventrikularne fibrilacije, sinusne tahikardije i srčanih blokova. Ipak, nastanak atrijalne fibrilacije (AF) kao posledice strujnog udara, dešava se retko. **Prikaz bolesnika.** Bolesnik, star 47 godina, primljen je u ambulantu nakon strujnog udara (< 600 V) koji je dobio. Nakon strujnog udara je izgubio svest, pao i zadobio povrede leđa i poglavine. Tokom pregleda, detektovana je aritmična akcija srca, ali bez šumova. Na levoj butini je registrovana ulazna, a na vratu izlazna rana. Elektrokardiogramom je registrovana novonastala AF. U urađenim laboratorijskim analizama nije bilo patološkog odstupanja, dok je ehokardiografskim pregledom registrovana očuvana kontraktilna snaga srca, bez poremećaja segmentne kinetike, uz normalne dimenzije leve pretkomore. Ordiniran je niskomolekularni heparin supkutano i 600 mg propafenona oralno. Nakon 24 h na elektrokardiogramu je registrovan normalni sinusni ritam. **Zaključak.** Prikazali smo redak slučaj AF uzrokovane električnom strujom koja je konvertovana u sinusni ritam primenom antiaritmika. Iako većina ovako nastalih epizoda AF predstavlja prolazni poremećaj srčanog ritma sa spontanom konverzijom, neophodno je rutinsko praćenje ovih bolesnika.

Ključne reči:
antiaritmici; fibrilacija pretkomora; lečenje lekovima; povrede električnom strujom; elektrokardiografija.

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia at discharge from hospital [1]. The etiopathogenesis of AF is rather complex and usually multifactorial [2]. Arterial hypertension, valvular heart disease, and heart failure are listed as the most common causes of AF. An electrical injury can cause various cardiac arrhythmias, such as asystole, ventricular fibrillation, sinus tachycardia, and heart blocks. However, it rarely causes AF [3]. We present a case of a patient with electrical injury-induced AF as a consequence of an occupational accident.

Case report

The 47-year-old patient was admitted to the Emergency Department of the Institute for Treatment and Rehabilitation "Niška Banja" because he was feeling palpitations, dizziness,

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and instability when standing and walking. The patient stated that he had come into direct contact with an exposed wire while using a pool cleaning machine, thus receiving an electric shock (< 600 V). He subsequently lost consciousness, fell, and sustained back and head injuries. He was unaware of how long he had been unconscious, but he felt irregular heart rate, dizziness, and exhaustion immediately after regaining consciousness. The patient confirmed that he was not suffering from any disease and had not been prior hospitalized or clinically examined. In addition, he denied the consumption of alcohol and the intake of medications and psychoactive substances.

During the examination, the patient was conscious, oriented, eupnoeic, and had normal skin color. There was an entry wound on the front of the left thigh and an exit wound on the front of the neck (Figure 1). There were no pathological findings in the lungs. His heart rate was irregular but with no heart murmurs. Vital parameters were as follows: blood pressure 120/80 mm Hg, heart rate 80 bpm, SaO_2 98%, body temperature 36.6 °C, and respiration rate 12 per min.

An electrocardiogram (ECG) showed previously nonexisting AF (Figure 2). The laboratory tests, which included cardiac-specific enzymes, troponin, electrolytes, complete blood analysis, and thyroid hormones, showed no pathological deviation. Focus cardiac ultrasound showed that contractile force was preserved with no wall-motion abnormalities and normal left atrium dimensions.

The patient was administered low-molecular-weight heparin (enoxaparin) subcutaneously and propafenone (600 mg) orally (pocket therapy). Wounds were treated, and an antibiotic was administered. At follow-up after 24 hrs, an ECG showed normal sinus rhythm (Figure 3). Once again, laboratory tests confirmed normal values. The patient was discharged in a stable state. A decision was made not to continue antiarrhythmic and anticoagulant therapy (CHA2DS2VASc score 0, HAS-BLED score 0).

Three months later, a 24 hrs ECG Holter monitoring showed normal sinus rhythm. Moreover, a follow-up cardiac ultrasound showed preserved ejection fraction, with neither valvular heart disease nor segmental wall-motion abnormalities, with the left atrium of 36 mm. A submaximal exercise stress test did not show signs of myocardial ischemia or arrhythmias.

Discussion

An electric shock can lead to myocardial necrosis, left ventricular dysfunction, arrhythmia, and conduction disorders [4]. The most common arrhythmias are sinus tachycardia, sinus bradycardia, ventricular fibrillation, and asystole, whereas the most prevalent conduction disorders are branch blocks, AV blocks of different degrees, and QT interval prolongation. On the other hand, it is quite rare for an electric shock to cause AF [5].

The mechanism of AF occurrence as a consequence of an electric shock is complex and only briefly examined.

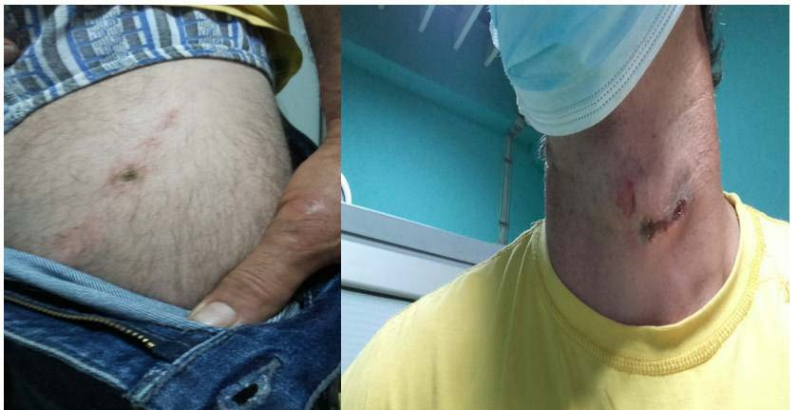

Fig. 1 – The entry wound on the left thigh and exit wound on the neck.

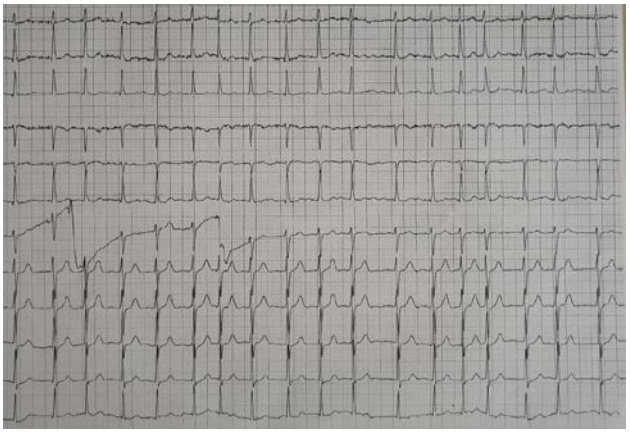

Fig. 2 – Electrocardiogram after the electrical injury.

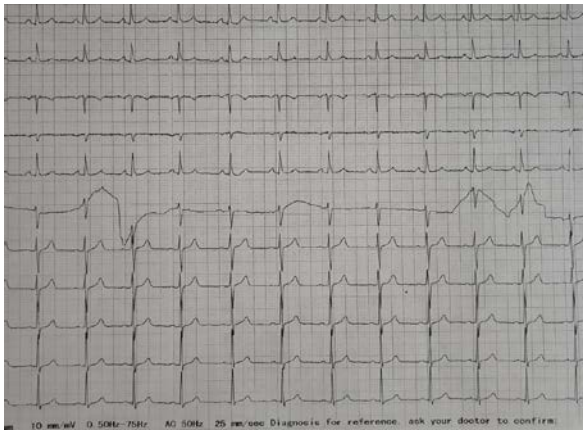

Fig. 3 – Electrocardiogram 24 hrs after the electrical injury.

Blood is an excellent conductor of electricity, and the heart is one of the most vulnerable organs when it comes to the impact of electric current. An electrical injury can lead to myocardial necrosis with subsequent fibrosis that may become a chronic arrhythmogenic focus [6]. Furthermore, an electric shock can disrupt the sodium-potassium pump with a subsequent increase in the concentration of potassium, which can potentially lead to a change in the permeability of cardiomyocytes and, therefore, to myocardial depolarization [7].

Other pathogenic mechanisms, such as coronary spasm, catecholamine release, and coronary hypoperfusion due to arrhythmia-induced hypotension are uncommon in the basis of the pathogenesis of electric shock-induced AF [8].

Which type of arrhythmia or conduction disorder exactly occurs as a consequence of an electric shock depends on the intensity of the electric current and its type (direct and alternating), the surface area of the body coming into contact with the electric current, the duration of the contact, and the state of the patient [9]. The changes caused by high voltage currents are usually complex and pronounced, whereas those caused by low voltage currents (< 600 V) are likely benign and transitory, which was the case with our patient. Most AFs after electric shocks are self-limiting [10], especially when caused by low voltage current [3]. Moreover, no AF relapse has been reported so far. However, Boggild et al. [6] reported a case of electric shock that caused AF for over 20 years, supporting the notion of chronic damage to the left atrium. That makes cardiac monitoring a necessary routine measure.

Various treatment methods have been developed to deal with electrical shock-induced AF but with a lack of proper guidance and protocols. These include DC cardioversion, pharmacological reversion, or even simple waiting for spontaneous resolution.

Conclusion

We report a rare case of an electrical injury-induced AF, which was converted to sinus rhythm by applying drug therapy. Although most cases of electrical injury-induced AF represent self-limited benign conditions, cardiac monitoring as a routine measure should be considered.

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CASE REPORT

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Unrecognized neuromyelitis optica spectrum disorder with pontine and *corpus callosum* microhemorrhage

Neprepoznati poremećaj spektra optičkog neuromijelitisa sa mikrokrvarenjem u ponsu i *corpus*-u *callosum*-u

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Abstract

Introduction. Neuromyelitis optica spectrum disorder (NMOSD) represents an immune-mediated neuroinflammatory syndrome, classified as a separate entity after the discovery of aquaporin-4 immunoglobulin G (anti-AQP4-IgG). The magnetic resonance neuroimaging spectrum of NMOSD classically consists of bilateral optic neuritis and longitudinally extensive transverse myelitis (LETM), recently broadened with lesions in *area postrema*, diencephalon, brainstem and cerebellum, and extensive cord atrophy. **Case report.** The case presents an anti-AQP4 autoantibody-positive 65-year-old female patient who initially presented with underestimated LETM and developed multiple cerebral and cerebellar lytic demyelinating lesions associated with acute long segment optic nerve involvement two years later. Two new imaging findings were described in this case: the involvement of a complete cross-sectional area of pons and microhemorrhage in the pons and *corpus callosum*. **Conclusion.** Raising suspicion of NMOSD is of crucial importance in cases with isolated LETM in order to prevent relapses in anti-AQP4-IgG positive cases and improve patient outcomes and recovery.

Key words:
anti-aquaporin 4 autoantibody; magnetic resonance imaging; neuroinflammatory diseases; neuromyelitis optica; treatment outcome.

Apstrakt

Uvod. Neuromijelitis optika – spektar poremećaja (NMOSP) predstavlja neuroinflamatorni sindrom, posredovan imunskim mehanizmima, klasifikovan kao poseban entitet nakon otkrića akvaporin-4 imunoglobulina klase G (anti-AQP4-IgG). Spektar nalaza NMOSP na snimcima magnetne resonance klasično uključuje bilateralni optički neuritis i longitudinalno ekstenzivni transverzalni mijelitis (LETM), a odskora je proširen lezijama u *area postrema,* dijencefalonu, moždanom stablu i cerebelumu, i ekstenzivnom atrofijom kičmene moždine. **Prikaz bolesnika.** Prikazana je bolesnica sa prisutnim anti-AQP4 autoantitelima, starosti 65 godina, sa inicijalnom, potcenjenom, prezentacijom LETM koja je razvila multiple cerebralne i cerebelarne litičke demijelinizacione lezije povezane sa akutnim neuritisom dugog segmenta optičkog nerva dve godine kasnije. Dva nova nalaza na snimcima opisana su kao zahvatanje kompletne transverzalne površine ponsa i mikrokrvarenja u ponsu i *corpus*-u *callosum*-u. **Zaključak.** Sumnja na NMOSP je od velikog značaja kod bolesnika sa izolovanim LETM da bi se sprečili relapsi u anti-AQP4-IgG pozitivnim slučajevima i poboljšala prognoza i oporavak.

Ključne reči:
anti-akvaporin 4 autoantitela; magnetna rezonanca, snimanje; bolesti, neuroinflamatorne; neuromijelitis optika; lečenje, ishod.

Introduction

Neuromyelitis optica spectrum disorder (NMOSD) represents an immune-mediated neuroinflammatory syndrome that became a separate entity after the discovery of aquaporin-4 immunoglobulin G antibodies (AQP4-IgG) [1].

The neuroimaging spectrum of NMOSD, classically consisting of bilateral optic neuritis and longitudinally extensive

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transverse myelitis (> 3 vertebral segments, LETM), has been broadened to include lesions in the area postrema, diencephalon, brainstem and the cerebellum, as well as longitudinally extensive cord atrophy as chronic sequelae [1]. Acute LETM spinal cord lesions are, however, the most specific neuroimaging characteristic of NMOSD [2]. Here we present a case of a 65-year-old female patient who initially presented with underappreciated LETM, with two new imaging findings described – the involvement of a complete cross-sectional area of the pons and hemorrhage in the pons and the splenium of the *corpus callosum*.

Case report

A 65-year-old female presented with lower back pain, spastic paraparesis, gait disorder, and urinary retention. Cerebrospinal fluid (CSF) was normal, and oligoclonal bands were negative both in CSF and serum. Magnetic resonance imaging (MRI) of the thoracic spine revealed an extensive, five segments long inflammatory process of the upper thoracic spinal cord mainly involving the central gray matter, misinterpreted as syringomyelia (Figures 1A and 1B).

Six months later, a follow-up MRI study revealed disease progression associated with cord edema and inflammation involving more than 8 segments in the thoracic spinal cord. Imaging of the brain revealed no abnormalities (Figures 1C and 1E).

Twenty-seven months later, the patient presented with altered consciousness, dysphagia, anarthria, and spastic paraplegia. Brain imaging showed bilateral, almost symmetric abnormalities in the periventricular white matter, *corpus callosum*, both corticospinal tracts at the level of the posterior limb of *capsula interna* (Figures 2A and 2B), mesencephalon, pons, superior and middle cerebellar peduncles and cerebellar white matter adjacent to the fourth ventricle, as well as in the ventral columns of the medulla oblongata. Diffusion was restricted at the periphery of the lesions. Susceptibility-weighted imaging (SWI) revealed discrete hemorrhage in the splenium of the *corpus callosum* and laterally in the pons. Contrast enhancement was vivid and heterogeneous (Figures 2C and 2D). At this point, a long segment of left optic nerve atrophy was pre-

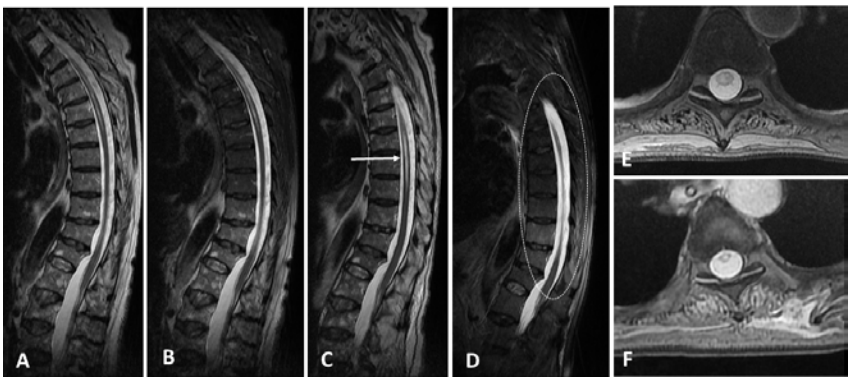

Fig. 1 – An extensive signal abnormality was evident in the upper thoracic spinal cord on the following: (A) T2-weighted and (B) STIR sagittal images, around 5 segments long, with significant cord swelling. A follow-up examination after 6 months (C) showed an extension of the lesion in the caudal direction (arrow) and (E) bright lesions around the central canal on the axial image. Two years later (D) extensive cord atrophy is registered on STIR sagittal (encircled) and (F) T2-weighted axial images. STIR – short tau inversion recovery.

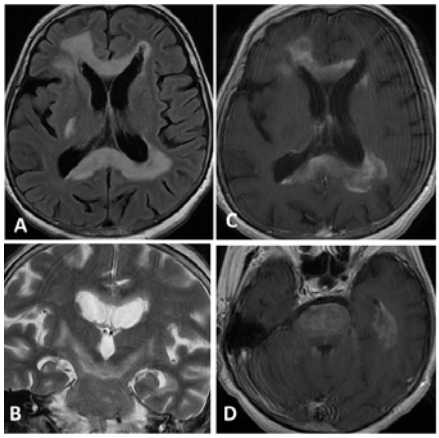

Fig. 2 – Extensive signal abnormality reflecting lytic demyelination was observed in the following: (A) periventricular white matter and *corpus callosum*; (B) affecting also both corticospinal tracts symmetrically suggestive of neuromyelitis optica spectrum disorder; (C, D) Vivid postcontrast enhancement was observed in the initial MR examination in corresponding areas. MR – magnetic resonance.

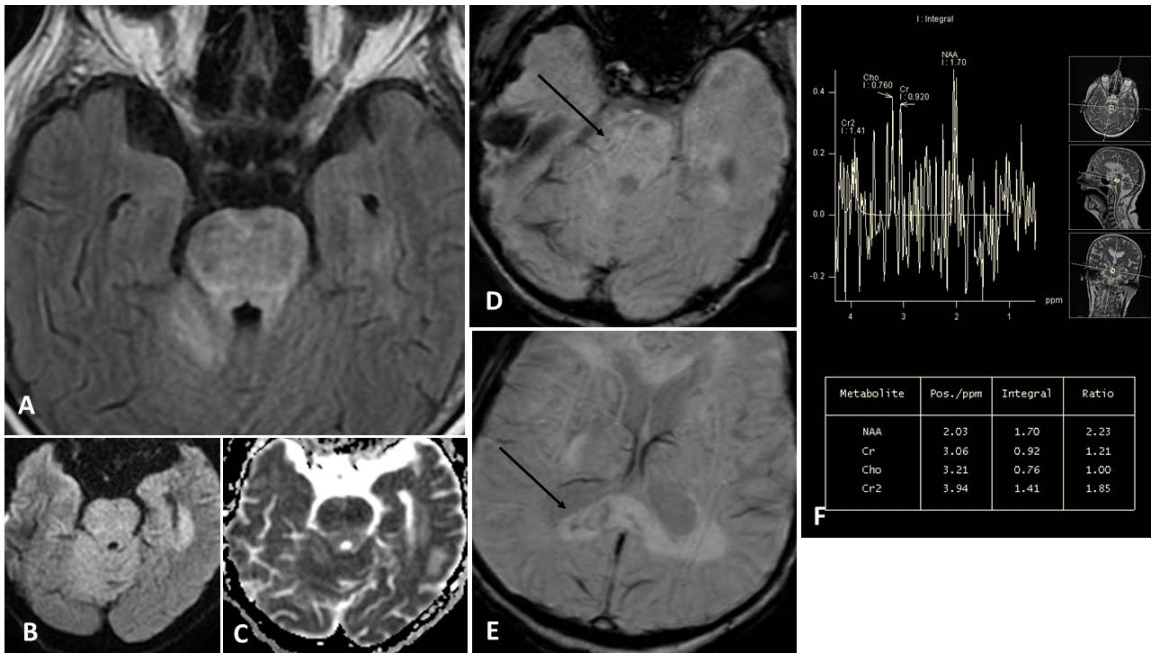

Fig. 3 – A lesion involving a complete cross-sectional area of the pons is evident on FLAIR axial image (A); showing signs of diffusion restriction − DWI image (B); (C) ADC map. Discrete signs of hemorrhage are observed in the right lateral aspect of the pons (D) and the right aspect of the splenium of the *corpus callosum* (E). Long echo time MR spectroscopy is of low quality, showing elevation in the Cho/Cr ratio, and a small lactate peak (F), implying the process of increased membrane metabolism and glial proliferation in inflammation.
FLAIR – fluid-attenuated inversion recovery; DWI – diffusion-weighted imaging; ADC – apparent diffusion coefficient; MR – magnetic resonance.

sent, with no contrast enhancement. Five days later, MR spectroscopy was performed in the pontine lesion (Figures 3A-3E), showing only an elevation of the Cho/Cr ratio and a small lactate peak compatible with anaerobic glycolysis in tumefactive demyelination (Figure 3F). The suspicion of NMOSD was raised, and lumbar puncture was repeated – CSF tested positive for AQP4-IgG. Follow-up imaging of the thoracic spine showed severe atrophy of the thoracic spinal cord and gliotic lesions surrounding the dilated central canal as sequelae of LETM (Figures 1D and 1F). The patient was initially treated with pulse doses of 1 g methylprednisolone for 7 days, which resulted in minimal neurological improvement. Due to an unsatisfactory response to treatment, plasma exchange was performed, followed by the introduction of immunosuppressive therapy with prednisone and azathioprine. The outcome was lethal.

Discussion

The diagnosis of NMOSD is based on both clinical and radiologic findings, according to the international consensus diagnostic criteria for NMOSD [1]. In this case, the patient was a 65-year-old female, and the disease followed a relapsing course in concordance with typical NMOSD epidemiologic findings [3]. Mortality rates are high in NMOSD, varying from 25–50%, and highly associated with neurogenic respiratory failure and extensive brainstem lesions [4].

The initial imaging finding in our patient was an isolated acute LETM, misinterpreted as a syrinx, with no concurrent brain lesions. LETM is typically considered one of the cardinal clinical findings in NMOSD, in conjunction with optic neuritis [5]. At the initial presentation, no signs of optic neuritis were evident, although it developed later during the disease. However, isolated myelitis as the only clinical manifestation is more common in male patients (67% vs. 28%) [6]. Follow-up MRI scans revealed the progression of LETM leading to severe and rapid atrophy of the affected spinal cord. A relapsing course was observed two years later with newly detected lesions in the brain, all classical NMOSD locations [1].

Spinal cord atrophy is considered a chronic manifestation of NMOSD. However, it usually develops over a longer period of time, up to 12 years [5]. It is suggested that spinal cord atrophy can potentially help differentiate between anti-AQP4 and anti-MOG positive patients, with anti-AQP4 patients having significantly more severe atrophy, which was true for our patient [5]. It must be noted that our patient was under no specific treatment for lesions in the spinal cord due to misinterpretation. Although modern therapy in concordance with the current guidelines was given (high-dosage methylprednisolone therapy for 3–5 days continuously, followed by plasma exchange as a rescue therapy option) [7], the outcome was lethal.

Previously reported brainstem lesions only accounted for focal lesions in the pons [8], while our patient presented with diffuse pontine lesions. This is the finding not so commonly observed in NMOSD patients, given that proposed patterns for brainstem lesions are focal and more

dorsally located [9]. One previously unreported finding was observed in our patient – a discrete linear hemorrhage in the right aspect of the pons and the splenium of the *corpus callosum*, visible on SWI. Kamo et al. [10] previously reported a case of major pontine hemorrhage, which was secondary to corticosteroid treatment and hypertension (202/127 mmHg). In our patient, the form of hemorrhage resembled that of microbleeds (Figures 3D and 3E), and no elevation of blood pressure was detected. This finding could be associated with corticosteroid therapy but might also represent the end-stage changes in demyelinating lesions [11].

Conclusion

Raising suspicion of NMOSD is essential in cases with isolated LETM, especially in cases of anti-AQP4 seropositivity, even when no lesions in the brain and optic nerves are present in order to prevent delay in diagnosis and improve patient outcome and recovery.

Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

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Reconstruction of the large columella defect with Schmid-Meyer flap

Rekonstrukcija velikog defekta kolumele *Schmid-Meyer*-ovim režnjem

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Abstract

Introduction. The reconstruction of columella defects is still regarded as a challenging procedure due to the very specific anatomy of the columella and limited local and regional flap options. Furthermore, the texture and color of columella tissue pose difficulties in choosing the right method of reconstruction. **Case report.** The report presents a patient who underwent reconstruction of a complex columella defect using a Schmid-Meyer flap. Schmid-Meyer flap represents a tubular flap with an internal supraciliary pedicle which allows the transposition of the temporal skin with the addition of ear cartilage on the tip of the nose or the *ala nasi*. The integration of the flap was complete. During the five-year follow-up period, the cosmetic and functional results were satisfying. **Conclusion.** Schmid-Meyer flap may be one of the best options for the reconstruction of complex defects of the columella.

Key words:
nasal septum; nose deformities, acquired; nose neoplasms; reconstructive surgical procedures; rhinoplasty.

Apstrakt

Uvod. Zbog vrlo specifične anatomije kolumele i ograničenog izbora lokalnih i regionalnih režnjeva, rekonstrukcija defekta kolumele i dalje predstavlja izazov. Isto tako, tekstura i boja tkiva kolumele predstavljaju poteškoće u odabiru prave rekonstruktivne metode. **Prikaz bolesnika.** U radu je predstavljen bolesnik kod koga je urađena rekonstrukcija složenog defekta kolumele pomoću *Schmid-Meyer*-ovog režnja. To je tubularni režanj sa vaskularnom peteljkom zasnovanom na supracilijarnim krvnim sudovima, koji dopušta transpoziciju kože temporalne regije sa graftom aurikularne hrskavice, na sam vrh nosa. Vitalnost prikazanog režnja je očuvana u potpunosti. Tokom jednogodišnjeg perioda praćenja, estetski i funkcionalni rezultati bili su zadovoljavajući. **Zaključak**. *Schmid-Meyer*-ov režanj može biti jedna od najboljih opcija za rekonstrukciju složenog defekta kolumele.

Ključne reči:
nos, septum; nos, stečene deformacije; nos, neoplazme; hirurgija, rekonstruktivna, procedure; rinoplastika.

Introduction

Up-to-date, various causes have been linked to columella defects, including congenital absence, trauma, and tumors. Reconstruction of these defects is a challenging procedure because of limited options for local and regional flaps and the main anatomic characteristics of this site. Several skin grafts, local and free flaps, have already been described in the literature, but none is recommended as the treatment of choice, which will ensure an excellent texture and color matching to the tissue [1].

Among many surgical techniques described to repair full-thickness defects of the inferior part of the nose, the Schmid-Meyer flap is one of the recommended [2, 3]. This flap is a tubular flap with an internal supraciliary pedicle which allows the transposition of the temporal skin and the addition of ear cartilage on the tip of the nose or the *ala nasi*. This little acclaimed technique is based on the old principle of autonomization of a cutaneous flap and uses a tailor-made composite cartilaginous graft placed in the flap. This meticulous reconstruction requires four stages which can be accomplished under local anesthesia [2, 3].

The main goal of this report was to present a successful reconstruction of a short and small columella using a Schmid-Meyer flap.

Case report

A 53-year-old male patient was admitted to our clinic with a tumor mass located in the columella region. On ini

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tial physical examination, the observed tumor involved the entire region of the columella, obstructing both external nares, and leading to the closure of the physiologic nasal pathway, without pain or bleeding. The excisional biopsy was performed, and a histopathologic examination of the obtained specimen showed squamocellular carcinoma, grade I. Total excision of the tumor was done with free surgical margins (Figure 1), and the arising defect was reconstructed with a Schmid-Meyer flap prefabricated with ear cartilage. The patient was followed up regularly for five years after the surgery and had no subjective complaints and no signs of recurrence of the disease.

Surgical technique

In the first stage, only the prefabrication was achieved. The piece of cartilage, 2.5 cm in length, was harvested from the right auricula and inserted into the pocket between the skin and frontal muscle at the distal site of the flap (right lateral superciliary region).

After one month, the patient underwent the second stage of the operation. After the right trochlear artery was identified with Doppler ultrasound, the lower limit of the flap was drawn close to the eyebrow margin (till its lateral border), and the upper limit of the flap was determined by a pinch test to allow for primary closure of the donor site. A partially tubular flap was made, including a cartilage graft with pedicled attachments on its proximal and distal points. The donor site has been primarily sutured (Figure 2).

The third stage, three weeks after the second stage, included the deinsertion of the flap (of its distal attachment) and suture to the lower side of the columella defect (columella base) (Figure 3). In the fourth stage, the final reconstruction was made. The flap was left for two weeks and then divided. After satisfactory perfusion of the flap was observed, the proximal attachment was divided and sutured to the upper side of the columella defect. The unused pedicle was sacrificed. The donor site was corrected (Figure 4).

Discussion

The columella links the nasal tip to the nasal base and separates the nares. It is composed of a pair of fine-textured cartilages and thin overlying skin. The columella, together with a pair of cartilages and caudal septum, provides necessary support and projection to the nasal tip [1]. The main problems in the reconstruction of columella are the following: its localization, narrow horizontal dimension, unique contour, its tenuous vascularity, and limited availability of adjacent tissue [4]. In line with the above stated, the use of different reconstructive techniques is limited and does not allow ideal reconstruction of columella defects [5]. Each technique has its own advantages and disadvantages, as described by the authors, and some require multiple operations. These techniques vary in complexity and include a wide range of operative procedures such as composite grafts [6], frontotemporal flap [2, 3], nasolabial flaps [7], naso-cheek flaps [8], buccal mucosal flap [9], free flap [4], forehead flap [10], and prefabricated flaps [11].

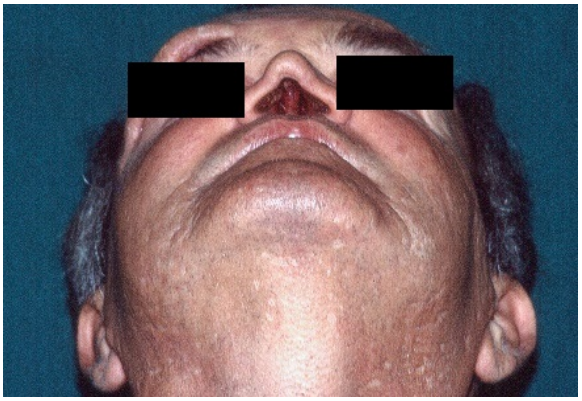

Fig. 1 – Post-excisional columella defect.

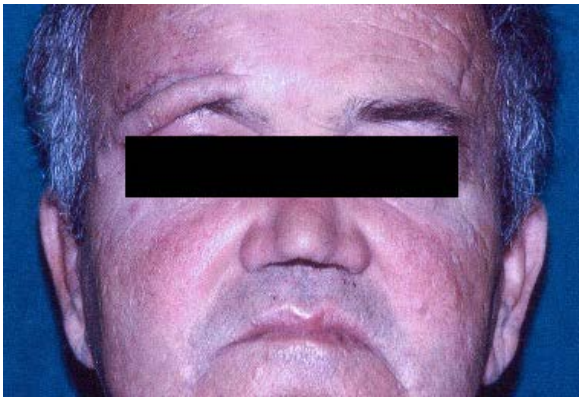

Fig. 2 – Partially tubular flap.

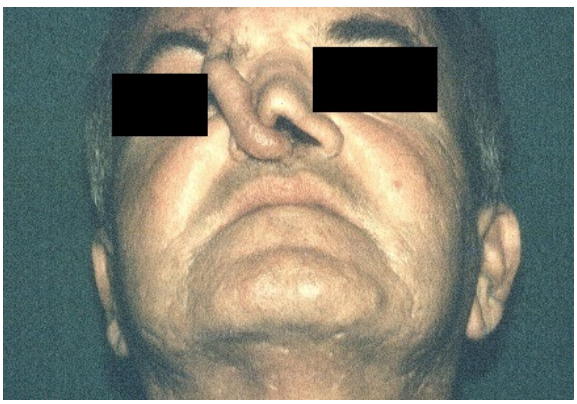

Fig. 3 – Columella defect coverage with Schmid-Meyer flap.

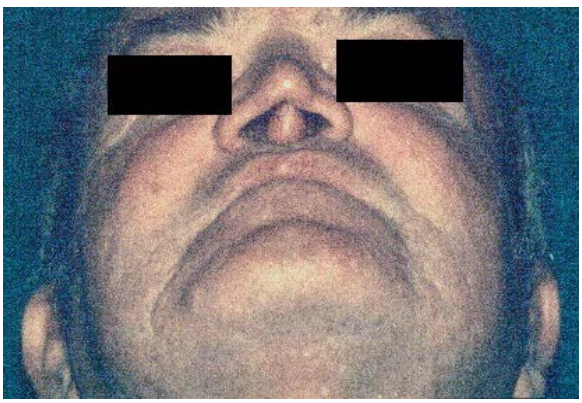

Fig. 4 – One year after the surgery.

The Schmid-Meyer frontotemporal flap is a tubular flap with an internal supraciliary pedicle that allows the transposition of the temporal skin with the addition of a tailor-made composite cartilaginous graft placed in the temporal region [12]. This graft is progressively detached, which allows repair of the lower third of the nose. Since its first description by Schmid in 1952 [2] and later modification by Meyer and Oppliger [3], the Schmid-Meyer flap has proved to be versatile for use in nasal reconstruction [13, 14]. In some specific indications, the Schmid-Meyer flap allows excellent reconstruction of the *ala*, the nasal tip, or the columella [14]. However, according to our knowledge, there have been only a few reports of this technique in columella reconstruction [14].

In this report, we showed that this procedure is very convenient for composite tissue defects such as columella. In our experience, the Schmid-Meyer flap procedure allows a nasal reconstruction of high quality due to its good color match, few forehead scarring sequelae, and minimal donor deformity. Cartilage in the flap of our patient ensured adequate support to the columella, and clinically obvious resorption was not observed after 5 years. However, this procedure has two main disadvantages. First, this flap is a little bulky for a columella, but if spontaneous atrophy does not occur, this could be solved by debulking procedures, as shown in our case. The second disadvantage was the fact that the procedure had four stages.

Conclusion

This report adds to previously published work on different techniques for columella reconstruction. That is noteworthy for several reasons. Firstly, our results indicate that the Schmid-Meyer flap can be regarded as an alternative among the surgical options for the reconstruction of columella defects because it achieves good and stable aesthetic results with a high-quality nasal reconstruction. Secondly, our report can allow comparisons between different reconstructive techniques used for columella defects. Finally, and more generally, it will help us understand this problem better and allow greater awareness of different possibilities in columella reconstruction.

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