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CONTENTS / SADRŽAJ

This year, European Cervical Cancer Prevention Week is celebrated from January 20 to January 26, in order to raise awareness among women about cervical cancer and ways of its prevention. Recognizing the importance of this campaign, Serbia has been actively participating in its celebration for the 13th time.

Ove godine, Evropska nedelja prevencije raka grlića materice obeležava se od 20. do 26. januara sa ciljem da se podigne svest žena u vezi sa rakom grlića materice i načinom za njegovo sprečavanje. Prepoznajući značaj ove kampanje, Srbija već trinaesti put aktivno učestvuje u njenom obeležavanju.

EDITORIAL

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Vojnosanitetski pregled – The year in review, 2019

Vojnosanitetski pregled – osvrt na 2019. godinu

Silva Dobrić

University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Belgrade, Serbia

As in previous years, in the first issue of the new volume of the Journal, an overview of the work of its Editorial Office and Editorial Board in the past year, with an emphasis on the main characteristics of the received and published articles is given.

As can be seen from Table 1, a total of 276 papers were received during the past year, most of which were, as usually, from the category Original article (221 or 80.1%), mainly from domestic authors (239 or 86.5%: 20.1% were from military medical institutions, mainly from the Military Medical Academy in Belgrade, and 79.9% from civilian medical and academic institutions). Particularly pleased is the fact that 13.4% of the received manuscripts were international ones, which is an indicator of the interest of authors from aboard to publish their papers in our journal.

Of the total number of manuscripts received in 2019, 21% were immediately rejected as ineligible, while, by the end of the year, another 12.7% of papers were rejected after the review process had been completed. By the end of 2019, 94 (34.1%) of the submitted manuscripts had received positive reviews from the reviewers and were accepted for publication, while 89 (32.2%) of the submitted papers are still in the reviewing process.

In the past year, a total of 182 articles from different categories were published in the Journal (Table 1). As before, most of them were from the category of Original articles (125 or 68.6%), and significantly less from the other categories (16.6% Case reports, 3.3% General reviews, 1.7% Current topics, etc.). In addition, in 2019, a total of 148 papers were published electronically as Online First with a DOI number being, and they will be published in one of the printed issues of the Journal in the coming period, after professional, linguistic and technical editing. At the moment, these papers are available to readers at the web-site of the Journal and via DOI Serbia database. As in previous years, the authors of the published articles were, in most cases, authors from Serbia. The number of published articles by foreign authors was also at the level of previous years (about 15%).

Table 1

Characteristics of received and published papers in Vojnosanitetski Pregled in 2019

Category of papers	Papers, n (%)	
	received	published
Editorial		1 (0.5)
Original articles	221 (80.1)	125 (68.6)
Case Reports	36 (13)	34 (18.6)
General Review		6 (3.3)
Current Topic	6 (2.2)	3 (1.7)
Practical Advice for Physicians	2 (0.7)	2 (1.2)
Short Communication		3 (1.7)
Preliminary Report	3 (1.1)	
History of Medicine	3 (1.1)	2 (1.1)
Letter to the Editor	5 (1.8)	3 (1.7)
Book Review		3 (1.7)
Total	276 (100)	182 (100)

After several years of successive increase of the Journal's impact factor (from 0.199 for 2010 to 0.405 for 2017), it dropped to 0.272 last year. Therefore, in the next period we have to make an extra effort to raise its value again, first of all, by accepting and publishing only high quality papers with topics that will arouse the interest of our readers and be cited by other authors. In achieving this goal I expect a lot of help from our editors and reviewers, because without their dedicated work, the Journal could certainly not exist.

At the end, I want to thank all of our authors, readers, and an eminent team of peer reviewers, editors and editorial staff that all collaborate to produce Vojnosanitetski Pregled.

The list of reviewers engaged in reviewing the papers in 2019 is given in Table 2.

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Table 2

Reviewers of Vojnosanitetski Pregled in 2019

Antić Ana	Gazivoda Dragan	Ljubić Aleksandar	Pešić Milica
Antonijević Nebojša	Genç A		Petakov Milan
Arsenijević Nebojša	Glišić Branislava	Magić Zvonko	Petronijević Milan
		Maksić Djoko	Petrova Genka
Baletić Nenad	Hrvačević Rajko	Mandić Gajić Gordana	Pljevljakušić Dejan
Balint Bela		Marić Nebojša	Počuča Milan
Bančević Vladimir	Igić Rajko	Marjanović Marjan	Polovina Snežana
Baščarević Vladimir	Ilić Tihomir	Marković Aleksa	Poštić Srđan
Bezmarević Mihajlo	Ivanović Mirjana	Marković Dejan	Pucar Ana
Bila Jelena		Martić Vesna	
Bogdanović Gordana	Jakovljević Mihajlo	Maslovarić Irina	Rabrenović Violeta
Bogdanović Jovo	Janjić Zlata	Matić Ivana	Radenković Dejan
Bokonjić Dubravko	Janjićijević Mirjana	Matijević Steva	Radenović Lidija
Bonaci Nikolić Branka	Janković Aleksandar	Mijušković Mirjana	Radoičić Dragan
Bouhani Malek	Janković Slobodan	Mijušković Željko	Radulović Danilo
Božović-Spasojević Ivana	Jauković Ljiljana	Mikić Dragan	Rančić Nemanja
Brdareski Zorica	Jelenković Popović Aleksandra	Mikov Aleksandra	Rasulić Rasulić
Brkić Zlata		Milenković Branislava	Resan Mirko
	Jiawei Zheng	Milenković Saša	Ristić Anđelka
Cerovac Nataša	Jokić Spasić Vesna	Milev Boško	Ristić Branko
Cikota Bojana	Jovanović Dragan	Milić Rade	Ristić Dragana
Cvetković Gordana	Jovanović Dragan	Milovanović Branislav	
Cvijanović Vlado	Jovanović Vesna	Milovanović Jovica	Savić Slobodan
	Jović Momir	Milovanović Tamara	Sekulić Vuk
Čolić Miodrag	Jović Stošić Jasmina	Mirković Darko	Simić Radoje
Čutović Tamara	Jović Vraneš Aleksandra	Mirković Ljiljana	Slavković Nemanja
	Jović Zoran	Mitić Igor	Stamenković Dragoslav
Ćuk Vladimir	Jovičić Bojan	Mladenović Irena	Stamenković Dušica
		Mladenović Zorica	Stanić Vojkan
Daković Dragan	Kalezić Nevena	Mrvić Petrović Nataša	Stankov Karmen
Davidović Lazar	Kandolf Sekulović Lidija		Stanković Nebojša
Dedić Gordana	Katanić Jasmina	Nedok Aleksandar	Stanković Nikola
Demeši Čila	Konstantinović Ljubica	Nešković Vojislava	Starčević Srđan
Dimitrijević Jovan	Koračević Goran	Nežić Duško	Stasević-Karličić Ivana
Dinčić Evica	Košević Branko	Nikolić Branka	Stefanov Rumen
Dinić Miroslav	Kovačević Aleksandra	Nikolić Dimitrije	Stepanović Petrović Radica
Dobić Silva	Kovačević Vojin	Nikolić Ljubiša	Stevanović Ivana
Dragičević Danijela	Kozomara Ružica	Ninković Milica	Stojanović Zvezdana
Dragojević Simić Viktorija	Krajnović Dušanka	Novaković Marjan	Stošić Srba
Dragović Tamara	Kronja Goran	Novaković Nenad	
Drulović Jelena	Kuhajda Ivan		Šobić Šaranović Dragana
	Kukić-Marković Jelena	Obradović Slobodan	Šubarević Vladan
Đukić Aleksandar		Ostojić Gordana	Šuljagić Vesna
Đukić Irena	Lajnert Vlatka		Šupić Gordana
Đenić Nemanja	Lakić Aneta	Pantic Igor	Šurbatović Maja
Đurić Dragan	Lalošević Dušan	Pejčić Tomislav	Šušnjar Snežana
Đorđević Snežana	Lavrnić Dragana	Pejović Milovančević Milica	
Đorđević Dragan	Lazić Zoran		Tadić Ivana
Đorđević Vladan		Perković Vučković Nataša	Tadić Vanja

Table 2 – *continued*

Tarabar Dino	Udovičić Ivo	Vojvodić Danilo	Wang Wen-Jie
Tepšić Ostojić Vesna	Urošević Ivana	Vojvodić Nikola	
Tiodorović Danica	Ušaj Slavica	Vučević Dragana	Zeba Snježana
Todorović Ljubomir	Ušjak Ljuboša	Vučićević Katarina	Zidverc Trajković Jasna
Todorović Milena		Vučinić Predrag	Zrnić Bogdan
Tomić Aleksandar	Vasilijić Saša	Vučinić Slavica	
Trifunović Zoran	Vasiljević Nađa	Vukomanović Aleksandra	Živković Slavoljub
Tušek Ivan	Velicki Lazar	Vukomanović Đurđević Biserka	Životić-Vanović Mirjana
	Vezmar Kovačević Sandra		

ORIGINAL ARTICLES

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Doppler middle cerebral artery peak systolic velocity measurement as diagnostic tool for fetal anemia after *in utero* transfusions in red blood cell alloimmunisation

Dopler merenje maksimalnog protoka u sistoli arterije cerebri medije kao dijagnostičko sredstvo u proceni fetalne anemije nakon intrauterine transfuzije kod bolesnica sa Rhesus aloimunizacijom

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Abstract

Background/Aim. Doppler sonography of fetal middle cerebral artery peak systolic velocity (MCA-PSV) can be used to predict fetal anemia and the need for *in utero* intravascular transfusion (IUIT) in red blood cell (RBC) alloimmunisation pregnancies. The aim of this study was to evaluate whether measurement of MCA-PSV in fetuses that had undergone one to three transfusions is a good diagnostic tool for fetal anemia. **Methods.** Study included 36 pregnancies treated due to RBC alloimmunisation in our tertiary referral center during the 5-year period (2012–2017). We measured MCA-PSV and hematocrit (Hct) in all patients. In seven pregnancies there was a need to perform sequential IUITs for correction of fetal anemia. In these patients we compared MCA-PSV and Hct values before and after every transfusion. **Results.** Hct and MCA-PSV correlated negatively before transfusion therapy (p = 0.035) and after the second transfusion (p = 0.046). Contrary, after the first (p = 0.954), before the second (p = 0.738), as well as before (p = 0.092) and after (p = 0,741) the third transfusions there were no significant correlations between Hct and MCA-PSV values. Hct values before and after transfusions were positively associated (p = 0.001), but MCA-PSV were not (p = 0.296). According to performed receiver operating characteristic (ROC) analysis the cut-off point of MCA-PSV for investigated patients was 1.22 multiples of its median (MoM). **Conclusion.** There is a reduction in MCA-PSV accuracy for assessing fetal anemia in previously transfused fetuses. Larger studies are needed to explain the reasons for these findings and potentially set new referral values of MCA-PSV for better diagnostics of fetal anemia.

Key words:
fetus; anemia; blood transfusion, intrauterine; blood group incompatibility; rh-hr blood group system; ultrasonography; hematocrit.

Apstrakt

Uvod/Cilj. Dopler sonografsko merenje maksimalnog protoka tokom sistole u fetalnoj arteriji cerebri mediji (MCA-PSV) može da se koristi u predikciji fetalne anemije kao i za određivanje potrebe za intrauterinom transfuzijom (IUIT) kod bolesnica sa Rhesus (Rh) aloimunizacijom. Cilj studije bio je ispitati da li je merenje MCA-PSV kod fetusa koji su prethodno imali jednu do tri transfuzije dobar dijagnostički pokazatelj za fetalnu anemiju. **Metode.** Ova prospektivna studija obuhvatila je 36 trudnica koje su ispitivane i lečene zbog Rh aloimunizacije u tercijarnom centru tokom petogodišnjeg perioda (2012–2017). Merili smo MCA-PSV i hematokrit (Hct) kod svih fetusa. Kod sedam trudnica bilo je neophodno primeniti intrauterino transfuzije radi korekcije anemije. Kod ovih bolesnica poredili smo vrednosti MCA-PSV i hematokrita pre i posle svake transfuzije. **Rezultati.** Hct and MCA-PSV su u negativnoj korelaciji pre terapije *in-utero* transfuzijom (p = 0,035) i pre i posle druge transfuzije (p = 0,046). Obratno, posle prve (p = 0,954), pre druge (p = 0,738), kao i pre (p = 0,092) i posle (p = 0,741) treće transfuzije nije nađena statistički značajna povezanost između vrednosti Hct i MCA PSV. Vrednosti hematokrita pre i posle transfuzije bile su u pozitivnoj korelaciji (p = 0,001), ali vrednosti MCA PSV nisu (p = 0,296). Prema ROC analizi granični skor za MCA PSV za ispitivane bolesnice bila je 1,22 MoM. **Zaključak.** Tačnost MCA PSV kao pokazatelja fetalne anemije redukovana je kod fetusa koji su

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prethodno imali transfuziju. Veće studije su potrebne da bi se objasnili uzroci ove pojave i da bi se potencijalno postavile nove referentne vrednosti MCA-PSV, a u cilju bolje dijagnostike fetalne anemije.

Ključne reči:
fetus; anemija; transfuzija krvi, intrauterina; krvne grupe, nepodudaranje; krvne grupe, rh-hr sistem; ultrasonografija; hematokrit.

Introduction

Red blood cell (RBC) alloimmunisation develops when a pregnant woman has an immunological response to a paternally derived red-cell antigen that is foreign to the mother but inherited by the fetus [1–3]. Maternal antibodies produced as immunological reaction can cross the placenta, bind to antigen present on the fetal erythrocytes and cause fetal anemia due to their hemolytic properties [1–3]. The treatment of choice for this condition is *in utero* intravascular transfusion (IUIT) of red blood cells that was first proposed by Liley [4]. A blood transfusion is indicated if fetal anemia is moderate (hemoglobin deficit 2–7 g/dL, i.e. < 0.65 multiples of its median – MoM) or severe (deficit greater than 7 g/dL, i.e. < 0.55 MoM) [5, 6].

The only absolutely accurate test to assess the degree of fetal anemia is fetal blood sampling. Another standard method for evaluation of fetal anemia is serial amniocentesis for the determination of bilirubin levels in amniotic fluid [7, 8]. Hemolysis leads to the accumulation of bilirubin in amniotic fluid, so its level correlates with the severity of hemolysis [9]. Spectrophotometry is used to quantify bilirubin level which is expressed as the change in optical density at a wave length of 450 nm. Those values are then plotted on a Liley chart to estimate the severity of anemia [4, 10]. However, both of these diagnostic procedures are invasive and consequently can cause miscarriage, premature rupture of membrane, preterm delivery as well as feto-maternal hemorrhage, thereby exacerbating the severity of the disease [11, 12].

Therefore, non-invasive methods for the prediction of fetal anemia and timing of its therapy have been assessed lately [6, 13]. Several studies have established that fetal anemia is associated with increased arterial and venous blood flow velocities. Fetal anemia causes decreased blood velocity leading to increased venous return and preload with consequent increase in cardiac output which is manifested as hyperdinamic circulation. These changes start occurring after the 16th gestational week when fetal reticulo-endothelial system is mature enough to destroy antibody-coated erythrocytes [1–3]. It is well known that hemodynamic changes can be assessed by Doppler ultrasound and therefore authors have proposed measuring the fetal middle cerebral peak systolic velocity (MCA-PSV) as the most accurate for potential prediction of alloimmunisation complications [9, 12].

Moreover, MCA-PSV is also generally used to decide when to perform IUIT and delivery. Nevertheless, there are still few studies regarding the non-invasive alloimmunisation diagnostics and their results regarding the reliability of MCA-PSV measurements are conflicting. The major issue presents the reliability of MCA-PSV for anemia diagnostics in previously transfused fetuses [7, 8].

Therefore, the aim of this study was to evaluate whether Doppler measurement of MCA-PSV of the fetus who has undergone one, two or three *in utero* transfusions due to correction of anemia, is a good diagnostic tool for timing next transfusion.

Methods

This prospective cohort study included all consecutive singleton pregnancies with RBC alloimmunisation that were checked-up, treated and delivered in our tertiary referral centre during the 5 year period (2012–2017).

Detailed history data were taken from every woman, such as age, BMI, obstetrical history and neonatal outcome for all previous pregnancies. Patients were regularly checked-up (laboratory and sonography) throughout pregnancy. After the 24th week of gestation examinations were performed every fortnight. We measured fetal MCA-PSV and hematocrit (Hct) in all patients.

The method of evaluation the MCA-PSV was classic. First, in transverse section of the fetal head we ultrasonographically identified the circle of Willis and the middle cerebral artery. Then, applying pulsed-wave Doppler on the proximal one-third of the MCA at the angle of < 20 degrees, three consecutive waveforms, in the absence of fetal body or breathing movements, were recorded. The highest level was considered as the PSV (cm/s). Finally, the PSV was recalculated in multiples of its MoM and this value was used in further analysis.

Cordocentesis was performed for fetal blood sampling and measurement of hemoglobin and hematocrit levels during pregnancy. Upon birth, blood sample from the umbilical cord was taken for laboratory testing.

In utero intravascular transfusion was indicated in cases of ultrasonographically suspected imminent or existing fetal hydrops (ascites and dilated heart) or whenever the optical index at 450 nm (DOD450) showed Liley zone B2 i.e. III. Fetal anemia was also considered if hemoglobin levels were < 7.0 g/dL and/or hematocrit < 30%. Moreover, severe fetal hemolysis was considered if antibody levels were > 35 IU/mL. If indicated transfusions were administered between the 28th, and 32nd gestational week in our study.

We compared MCA-PSV and Hct values before and after every transfusion and assessed their relationship. The extent of MCA-PSV change after IUIT treatment was calculated. Finally, we assessed the impact of MCA-PSV and Hct values before and after every transfusion on neonatal outcome.

As main neonatal outcomes of the examined pregnancy we considered gestational age at birth, birth weight and Ap-

gar score. We also measured hemoglobin and hematocrit levels at birth for every live-born child.

Obtained data were statistically analyzed by methods of descriptive and analytical statistics (percentages, mean, median, standard deviation, ANOVA, Wilcoxon Z test, Kruskal Wallis χ^2) and a computer program SPSS 20. Spearman correlation was applied to assess the relationship between parameters of fetal condition before and after transfusion treatment. To examine to which extent MCA-PSV measurement can be used to differentiate the fetal anemia patients and cases that do not need to have IUIT we calculated the tests accuracy (number true positives + true negatives/all examined patients number). As the cut-off level we used the generally accepted 1.5 MoM MCA-PSV. Finally, we performed the receiver operating characteristic (ROC) analysis to assess the best cut-off values of MCA-PSV timing first, second and third IUIT based on Hct values.

Results

Study included 36 pregnant women with RBC alloimmunisation. The mean age of these women was 31.14 ± 5.49 years. In their previous pregnancies two women had already had RBC alloimmunisation. In the monitored pregnancy IUIT for correction of fetal anemia had to be performed in seven cases. There were significant differences in the second trimester MCA-PSV (p = 0.016) and Hct (p = 0.020) between patients who needed and those who did not need IUIT. In one case we registered the presence of different antibody types while the remaining six patients had only RhD antibodies in the examined pregnancy. According to Liley score four women were in A, i.e. I zone, two in B1, i.e. II and one had B1/B2, i.e. II/III Liley zone.

We registered fetal hydrops in four cases and polycythemia in one case of the seven pregnancies that needed IUIT for fetal anemia treatment. Investigated patients were delivered in average in the 35th gestational week (34.5 ± 0.87 weeks). The mean birth-weight of their children was 2633.33 ± 246.64 grams while their Apgar score was quite low ranging from 1 to 7 (4.5 ± 2.38). Average hemoglobin level upon birth was 14.7 ± 1.5 g/dL (minimum 13.6, maximum 16.4 g/dL) indicating adequate treatment of fetal anemia. Still, we had one case with adverse pregnancy outcome in which IUIT did not mend the hemolysis.

Transfusion was performed three times in four and four times for remaining three patients. We presented the parameters of fetal condition (Hct and MCA-PSV) before and after every IUIT in Table 1. There were significant differences between levels of both Hct and MCA-PSV before and after the completion of transfusion treatment. The Hct levels were increased almost twice after administered IUITs (Table 1).

We also examined the relationship of Hct and MCA-PSV before and after each of the administered transfusions (Tables 2 and 3). It can be seen that levels of Hct and MCA-PSV (MoM) negatively correlated before the commencement of transfusion treatment as well as after the first and second transfusions. However, before the second as well as before and after the third transfusions there were no significant correlations between Hct and MCA-PSV (MoM) values. This can be explained by reduction in accuracy of MCA-PSV for assessing fetal anemia in previously transfused fetuses.

Table 1
Investigated parameters of fetal condition (Hct and MCA PSV) in pregnancies with red blood cell alloimmunisation before and after IUIT

Parameters	Min	Max	Mean ± SD	*Z*	*p*
Hct before transfusion	0.10	0.50	0.27 ± 0.09	-5.234	0.001
Hct after transfusion	0.26	0.55	0.42 ± 0.07		
MCA PSV MoM before IUIT	0.62	1.60	1.17 ± 0.24	-4.577	0.001
MCA PSV MoM after IUIT	0.44	1.07	0.79 ± 0.16		

Hct – hematocrit; IUIT – *in utero* intravascular transfusion; MoM– multiples of median, MCA-PSV – fetal middle cerebral artery peak systolic velocity; Min – minimum; Max – maximum; SD – standard deviation.

Table 2
Correlations of Hct and MCA-PSV before each of the administered transfusions

Parameters		MCA-PSV MoM before transfusion I	MCA-PSV MoM before transfusion II	MCA-PSV MoM before transfusion III
Hct before transfusion I	Ro	-0.929	-0.414	-0.290
	p	0.003	0.355	0.577
Hct before transfusion II	Ro	-0.378	-0.345	-0.809
	p	0.403	0.448	0.051
Hct before transfusion III	Ro	-0.609	-0.265	-0.088
	p	0.200	0.612	0.868

Ro – Spearman`s coefficient of correlation.
For other abbreviations see under Table 1.

Table 3

Correlations of Hct and MCA-PSV after each of the administered transfusions

Parameters		MCA-PSV MoM before transfusion I	MCA-PSV MoM before transfusion II	MCA-PSV MoM before transfusion III
Hct before transfusion I	Ro	-0.893	-0.179	-0.600
	p	0.007	0.702	0.208
Hct before transfusion II	Ro	0.631	-0.912	0.290
	p	0.129	0.011	0.577
Hct before transfusion III	Ro	-0.232	-0.696	-0.348
	p	0.658	0.125	0.499

For abbreviations see under Tables 1 and 2.

Finally, we determined the MCA-PSV measurement accuracy prior to IUIT treatment in detecting fetal anemia. For our sample it was rather low – 14.29%. So, it can be seen that the indication for IUIT has to be made in accordance with laboratory testing and not only MCA-PSV measurements.

Performed ROC analysis shows that MCA-PSV (with standard cut-off level on 1.5 MoM) adequately predicts 83.3% of fetal anemia cases prior to transfusion therapy. On the other hand, in our sample MCA-PSV predicts 50% before second transfusion and only 25% of anemic fetuses before the third transfusion. For investigated pregnancies the optimal MCA-PSV cut-off for timing the second as well as the third transfusion was 1.22 MoM (II IUIT sensitivity = 100%; specificity = 50%; III IUIT sensitivity = 100%; specificity = 33.3%).

Discussion

In the clinical management of RBC alloimmunization the major concern is to identify the affected fetus and to correct the fetal anemia by intrauterine blood transfusion, on time [1, 14]. The timing of IUIT is important because each procedure bears a 1.5–3% risk of fetal morbidity and mortality. The adequate evaluation of fetal anemia severity should enable clinicians to avoid unnecessary interventions. However, when to perform a subsequent transfusion is a subject of ongoing debate [8, 15].

There are several methods for timing of the first, second, and subsequent transfusions. Some centers still perform IUIT empirically every 7–10 days for gestations ≤ 24 weeks, 15 days for subsequent transfusions after the second one or 21 days once fetal erythropoiesis has been suppressed [11, 16]. Others use the decline in fetal hemoglobin of 0.4, 0.3 and 0.2 g/dL/day for calculating the intervals between first, second and third transfusion [4, 17]. It is known that after sequential transfusions decrease in hemoglobin concentration becomes almost regular at about 1% hematocrit point per day due to suppression of fetal red cell production and predomination of donor adult blood cells [5]. However, if fetal hydrops occurs, decline in fetal Hct is more rapid mostly requiring a shorter period between the transfusions [16].

Currently, it is widely accepted that MCA-PSV is not only accurate test for fetal anemia prediction before first transfusion treatment, but that it is also useful for timing subsequent transfusions [10, 18, 19]. The reliability of MCA-PSV in detecting fetal anemia was established 17 years ago in the initial report by Mari et al. [1] which gave a normative data for gestational age and threshold values for predicting moderate to severe anemia. Other investigators determined that accuracy of MCA-PSV Doppler assessment is better (85%), than the Liley curve (76%) and the Queenan curve (81%) [8]. These data have led to acceptance of using Doppler assessment of MCA-PSV in detecting fetal anemia in RBC alloimmunization as the primary diagnostic tool [6, 20]. When the critical antibody titer above 32 is detected, Doppler assessment of MCA–PSV should be performed serially every 1–2 weeks [14, 15]. Literature data have proved the association of MCA–PSV and fetal hemoglobin in fetuses before and after first transfusion [13, 20]. It is mostly accepted that MCA–PSV greater than 1.5 MoM is an indication for cordocentesis for fetal hematocrit determination and decision whether IUIT is needed [1, 2, 9].

However, cut-off levels of MCA-PSV for diagnosis of fetal anemia following serial transfusions have not yet been precisely defined [16, 17]. According to some literature data the overall performance of MCA-PSV in predicting severe anemia is almost unchanged from the first to the last IUIT, suggesting the usefulness of MCA-PSV in cases of serial transfusions [10, 21]. Conversely, other investigations found that reliability of MCA-PSV in serial transfusions is decreased necessitating higher cut-off levels upon which a decision to perform IUIT should be made [13, 20]. The reasons for a decreased predictive value of MCA-PSV following IUIT are still not completely understood, but relate with lower fetal blood viscosity after IUIT. This can be explained by changing fetal blood with donor adult red cells, which, when compared to fetal red cells, are smaller, have decreased cellular rigidity and increase erythrocyte aggregation [17, 22]. After couple of IUITs most of the circulating red cells in the fetal circulation are donor cells that contain adult hemoglobin which can decrease the delivery of oxygen at the fetal tissues. This could affect the cerebral vascular regulation and account for increases in MCA-PSV [19]. Moreover, IUITs are associated with higher fetal hematocrit levels, which consequently increases the whole blood viscosity [14, 15]. Both of these will slow the speed at which blood moves through the fetal circulation. Thus, it was demonstrated that detection rate of MCA-PSV with cut-off for anemia set on 1.5 MoM does not go above 64% [10, 18].

Therefore, as the positive predictive value of MCA-PSV decreases with each subsequent IUIT numerous authors believe that modified threshold for detecting moderate-severe anemia should be used. Still, results on the new cut-off levels are conflicting and while some studies report increased cut-off for transfusion (> 1.7 MoM) others propose to use the decreased levels (1.2 to 1.3 MoM) of MCA-PSV when deciding about the second and/or third IUIT [15, 19].

It is still not certainly whether transfusion interval between the second and the third IUIT can be accurately assessed by MCA-PSV and more studies on larger samples are needed [11, 15]. Therefore, in literature several normal reference ranges of MCA-PSV are mentioned and it is thought that any of them could be used if cut-offs are reviewed and patients serial measurements are monitored on an individual basis [2, 5]. Still, other investigations indicate that discriminatory power, sensitivity and specificity of Mari's curve and its given cut-offs are still the optimal for prediction of fetal anemia [10, 21].

We found that MCA-PSV and fetal Hct are significantly correlated before and after the first and after the second IUIT. However, before the second as well as before and after the third IUIT, in our study, there was no association of MCA-PSV and fetal Hct. These findings correspond to those reported in some previous studies that the MCA-PSV in not completely reliable in predicting severe anemia in fetuses that already had two previous transfusions [3, 8, 9, 20]. Moreover, our findings support the application of decreased cut-off MCA-PSV levels for timing subsequent transfusions. We suggest that the use of 1.22 MoM threshold in our population should be reconsidered.

The main limitation of our study is the very small sample. The available data do not allow us generalizing specificity of MCA-PSV assessment in the diagnosis of fetal anemia after two or more transfusions. However, RBC alloimmunisation nowadays is quite rare condition due to adequate prevention. The incidence of red blood cell alloimmunisation in literature is only 0.6%. There are even less fetuses that have such severe anemia that they need the transfusion treatment. Nevertheless, RBC alloimmunisation is not only extremely important, but also very current as it is still both diagnostic and therapeutic challenge. Consequently, other studies have also been performed on 10 cases, which for this rare condition is considered as an adequate sample and any study of RBC alloimmunisation presents valuable data for perinatologists. Therefore, we wanted not only to present a case-series of our patients, but also to statistically analyze obtained data and give some new perspectives on the condition. Still, we do believe that further studies on more patients or potentially some meta-analyses should be performed to test the accuracy of the newly established MCA-PSV cut-off for our population and make more reliable statistical conclusions.

Conclusion

In this study we proved that there is a reduction in accuracy of middle cerebral artery peak systolic velocity for assessing fetal anemia in previously transfused fetuses. Therefore, Doppler measurement of middle cerebral artery peak systolic velocity of the fetus who has undergone two or more *in utero* transfusions for anemia correction cannot be the only diagnostic tool for timing of serial transfusions. It has to be assessed together with the mean projected daily decrease in fetal hemoglobin. We suggest that new cut-off levels should be created that might enable better accuracy of middle cerebral artery peak systolic velocity in prediction of fetal anemia severity. Accordingly, further studies on larger samples are needed for such calculations.

Conflict of interest

Authors declare no conflict of interest.

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Health consequences of domestic violence against women in Serbia

Zdravstvene posledice porodičnog nasilja nad ženama u Srbiji

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Abstract

Background/Aim. Domestic violence against women is a significant public health problem resulting in serious health and social consequences, for women and their families. The aim of this study was to analyze the sociodemographic characteristic of women who were exposed to domestic violence, as well as the impact of violence on women's health. **Methods.** Data from cross-sectional study from the 2013 National Health Survey in Serbia were used analyzing 6,320 women aged 20–75 years. Univariate and multivariate logistic regression analyses were implemented to assess the association of exposure to domestic violence against women with sociodemographic characteristics, as well as with selected health indicators and health risk behaviors. **Results.** Out of total number of examined women, 307 (4.9%) reported that they experienced physical and/or psychological violence in the last 12 months. Divorced or separated women, poor women and women with poor social support had greater odds for exposure to domestic violence. Women who had experienced domestic violence were less likely to perceived their health as good than women who had not experienced domestic violence [adjusted odds ratio (AOR) = 0.47; 95% confidence interval (CI) = 0.32–0.71], and more likely to report severe or very severe pain (AOR = 2.41; 95% CI = 1.74–3.33), stress and pressure exposure (AOR = 2.62; 95% CI = 1.89–3.64) and depression (AOR = 3.24; 95% CI = 2.08–5.03). Exposure to violence was also associated with the use of sleeping pills or sedative (AOR = 2.21; 95% CI = 1.67–2.93), with frequent use of alcohol (AOR = 1.42; 95% CI = 1.08–1.86) and abortion (AOR = 3.11; 95% CI = 1.48–6.54). **Conclusion.** Women, victims of domestic violence are more likely to have physical and mental disorders compared to women who are not victims of domestic violence. Violence prevention demands a multisectoral approach, in which the health sector has a central role that includes early identification and recognition of abuse, appropriate care as well as documenting and reporting violence.

Key words:
battered women; domestic violence; health; risk factors; socioeconomic factors; women.

Apstrakt

Uvod/Cilj. Nasilje nad ženama u porodici je značajan javno-zdravstveni problem koji ostavlja ozbiljne zdravstvene i socijalne posledice po žene i njihove porodice. Cilj rada bio je da se analiziraju sociodemografske karakteristike žena koje su bile izložene porodičnom nasilju, kao i uticaj nasilja na zdravlje žena. **Metode.** Za potrebe istraživanja korišćeni su podaci Nacionalne studije istraživanja zdravlja stanovnika Srbije iz 2013. godine koja predstavlja studiju preseka na uzorku od 6 320 žena starosti 20–75 godina. Povezanost izloženosti nasilja sa socio-demografskim karakteristikama žena kao i sa zdravstvenim indikatorima i faktorima rizičnog ponašanja analizirana je korišćenjem modela univarijantne i multivarijantne logističke regresije. **Rezultati.** U toku poslednjih 12 meseci, 307 (4,9%) žena je bilo izloženo fiičkom i/ili psihičkom nasilju u porodici. Razvedene i razdvojene žene, siromašne žene, kao i žene sa slabom socijalnom podrškom, češće su bile izložene nasilju. Žene koje su bile žrtve nasilja u porodici ređe su procenjivale svoje zdravlje kao dobro u odnosu na žene koje nisu bile žrtve nasilja u porodici [*adjusted odds ratio* (AOR) = 0,47; 95% *confidence interval* (CI) = 0,32–0,71], češće prijavljivale teške ili jako teške telesne bolove (AOR = 2,41; 95% CI = 1,74–3,33), izloenost stresu i pritisku (AOR = 2,62; 95% CI = 1,89–3,64) i bile depresivne (AOR = 3,24; 95% CI = 2,08–5,03). Žene žrtve nasilja u porodici češće su koristile lekove za spavanje ili za smirenje (AOR = 2,21; 95% CI = 1,67–2,93), alkohol (AOR = 1,42; 95% CI = 1,08–1,86) i češće su imale namerne prekide trudnoće (AOR = 3,11; 95% CI = 1,48–6,54) u odnosu na žene koje nisu bile žrtve nasilja u porodici. **Zaključak.** Žene žrtve nasilja pokazuju češće poremećaje u sferi fizičkog i psihičkog zdravlja u odnosu na žene koje nisu žrtve nasilja u porodici. Prevencija nasilja zahteva multisektorski pristup, u

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kome zdravstveni sektor ima centralnu ulogu koja podrazumeva ranu identifikaciju i prepoznavanje nasilja, adekvatnu pooć, kao i dokumentovanje i prijavljivanje nasilja.

Ključne reči:
nasilje nad ženama; nasilje, porodično; zdravlje; faktori rizika; socijalno-ekonomski faktori; žene.

Introduction

Violence against women is a significant public health problem, as well as a fundamental violation of women's human rights. According to the World Health Organization (WHO) domestic violence is one of the most widespread forms of violence against women. The prevalence is high, and there are serious physical and mental health, as well as social consequences, for women and their families [1].

Domestic violence is defined as "all acts of physical, sexual, psychological or economic violence that occur within the family or domestic unit or between former or current spouses or partners, whether or not the perpetrator shares or has shared the same residence with the victim" [2]. Victims of domestic violence are mainly women, but also other family members such as children and elderly. The perpetrator is a part of the victim's domestic environment: husband, intimate partner, former intimate partner, family member or friend [3]. However, the most common form of domestic violence is intimate partner violence (IPV) against women [1, 4, 5].

A 2013 analysis conducted by WHO in over 80 countries, showed that almost one third (30%) of all women who have been in a relationship have experienced physical and/or sexual IPV. The prevalence ranges from 23.2% in high-income countries to 37% in the Eastern Mediterranean region and the South-East Asia region. Globally, as many as 38% of all murders of women are committed by their intimate partners [1].

IPV against women can be analyzed either as psychological, physical, sexual, violence, or any combination of these [3]. Terms: "domestic violence", "intimate partner violence", "battering", "wife/spouse/partner abuse" are often used interchangeably [6, 7]. Domestic violence does not describe a single violent event, but rather a complex system of abuse that has adverse consequences to women's health and well-being of children. Being exposed to family violence as a child presents an important risk factor for later engagement in unhealthy behaviour, as well as in perpetrating or experiencing violence in adulthood [8,9].

The WHO acknowledges the association between violence and adverse consequences for health, as shown in numerous studies conducted worldwide [1, 5]. IPV has been associated with several short and long-term health consequences including injuries, mental health problems, substance and alcohol abuse, psychosomatic diseases, noncommunicable diseases, sexual and reproductive health disorders (including unwanted pregnancy and abortion) and death (homicide or suicide) [1, 10–12].

Domestic violence demands serious monitoring especially in countries in transition that face the consequences of previous wars and economic crises such as poverty, unemployment, social insecurity and rises in violence in society. The situation of this problem remains largely unrecognizable due to the lack of routine data collection, causing the absence of necessary intervention by the society [13]. Domestic violence is considered a hidden problem associated with social stigma, self-blame, fear from perpetrator and acceptance as a norm in some societies [4, 14, 15]. Women who have been victimized find it hard to share their experiences and seek help. Physicians are often the first contact persons in case of domestic violence. Therefore, it is important that medical personnel is trained to identify abuse early, providing victims with the necessary treatment, and referring women to appropriate and informed care [3, 4, 10]. The aim of this study was to analyze the socio-demographic characteristic of women who were exposed to domestic violence, as well as the impact of violence on women's health.

Methods

Study design and sampling

Data for this study were obtained from the 2013 National Health Survey of the Serbian population (without Kosovo and Metohia), carried out by the Ministry of Health of the Republic of Serbia. A stratified two-stage sample was used to provide statistically reliable estimate of a larger number of variables that indicate the health of a population. In the first stage, a total of 670 census circles were selected. The second stage units were selected from the household list (10 households and three reserve) using the simple random sample without replacement. Out of total, 10,089 households were randomly selected and 6,500 of them agreed to participate in the survey (response rate 64.4%). Of 16,474 registered household members, aged 15 years and over, 14,623 were interviewed giving a response rate of 88.9%. Ethical standards applied in this study were in compliance with the international standards, Helsinki Declaration (World Medical Association Declaration of Helsinki) and Directive of the European Parliament on Protection of Individuals with regard to the Processing of Personal Data (Directive 95/46/EC), and specific legislation in Serbia. All respondents were informed about the purpose of the study and agreed to participate. Three types of questionnaires were used: self-administered questionnaire, face-to-face questionnaire and household questionnaire [16]. Out of total, 13,756 respondent completed questionnaires giving a response rate of 94.1%. For the purpose of this study we analyzed data on 6,320 women aged 20–75 years.

Study variables

Women were considered exposed to domestic violence if they reported having experienced physical and/or psychological violence in the last 12 month. Domestic violence was determined from two following questions: "Were

you exposed to any physical violence in the family during the last 12 months?" and "Were you exposed to any psychological violence (insults, humiliation, contempt, mockery, extortion) in the family during the last 12 months?" Variables included sociodemographic characteristics: age, type of settlement, marital status, education, material status (Wealth Index), employment status and social support. According to the Wealth Index (Demographic and Health Survey Wealth Index) respondents were classified into three socioeconomic groups or quintiles: rich (richer and the richest class), middle and poor (poorest and poorer) [17]. Employment status was divided into two categories: employed and unemployed/inactive (retired, students, housewives, unable to work and other inactive). Social support was measured using the Oslo-3 Social Support Scale (OSS-3) with three questions. It covers different fields of social support by measuring the number of people, the respondent feels close to, the interest and concern shown by others, and the case of obtaining practical help from others. The OSS-3 scores were divided into three categories: poor support (score 3–8), moderate support (score 9–11) and strong support (score 12–14) [18]. All health-related indicators were self-reported, and only ever-partnered women (women who were ever married or lived with a partner) were included in this part of the analysis. Self-perceived health was grouped into three categories: good (very good or good), fair, and poor (poor or very poor). Women were asked whether they experienced in the last 4 weeks: pain (no pain/mild pain, moderate pain, severe/very severe pain), and whether they felt tense or stressed/under pressure. To assess the presence of depressive disorders in the last 2 weeks the eight-item The Patient Health Questionnaire depression scale (PHQ-8) was used. A total score of 0 to 4 represents no significant depressive symptoms; score of 5 to 9 represents mild depressive symptoms; and score ≥ 10 represents depression [19]. Alcohol consumption in the last 12 months was categorized as frequent (once a week or more frequent), moderate (2–3 times per month or less), and non-drinkers (did not consume). Women were also asked to confirm or decline whether they were using sleeping pills or sedatives (no, uses sleeping pills or sedatives, uses both types of pills) and whether they had an abortion in the last 12 months.

Statistical analysis

Univariate and multivariate logistic regression analyses were used to assess the association of exposure to domestic violence against women with sociodemographic factors. Selected health indicators and risk behavior among women according to exposure to domestic violence were first examined using χ^2 tests, then, in order to determine the impact of domestic violence as an independent variable on health, univariate as well as seven multivariate logistic regression models was implemented. Dependent variables (health condition and risk behavior) were transformed into dichotomous variables. Models included eight independent variables: domestic violence, age, type of settlement, marital status, education, wealth index, employment status and social support. Data was weighted in order to be more representative for the Serbian population in 2013. We calculated the association through odds ratio (OR) with 95% confidence intervals (CI). The probability, $p < 0.05$, was taken as the minimum level of significance. All the statistical analyses were performed with the SPSS, 21.0 statistical package.

Results

Out of the total number examined women 307 (4.9%) reported that they experienced physical and/or psychological violence in the last 12 months (1.4% were exposed to physical and 4.4% to psychological violence). However, 432 (6.8%) women did not answer the questions about domestic violence and response rate was 93.2%.

The sociodemographic characteristics of women who were exposed to domestic violence in the last 12 months are shown in Table 1. The results of the univariate logistic regression analysis indicated that the exposure to domestic violence was neither associated with women's age nor with type of settlement. However, domestic violence was significantly associated with marital status, as well as education, material status, employment status and social support. A multivariate logistic analysis showed the consistency of the association of marital status, material status and social support with exposure to domestic violence among women, while association with education and employment status could not be shown. Divorced or separated women were more likely to be exposed to domestic violence (OR = 2.97; 95% CI = 1.66–5.31) compared to women who never married or never lived with a partner and the odds were higher than for married women (OR = 1.90; 95% CI = 1.19–3.04). Poor women were more likely to be exposed to domestic violence compared to rich ones (OR = 1.58; 95% CI = 1.13–2.19) and women with poor social support compared to those with strong social support (OR = 2.54; 95% CI = 1.77–3.64).

Ever-partnered women who were exposed to domestic violence in the last 12 months, reported significantly more health problems and risk behaviors than women who were not exposed to violence (Table 2). Every fourth (25.5%) woman exposed to violence perceived their health as poor ($p < 0.001$), and every third (31.3%) woman reported that in the last 4 weeks had severe or very severe pain ($p < 0.001$). In the last 4 weeks, 83.4% of women exposed to violence were under stress or pressure ($p < 0.001$), 23.3% had mild depression symptoms and 12.0% had depression ($p < 0.001$). More than one-third (36.0%) of woman exposed to violence used sleeping pills or sedatives in the last 12 months, and 14.3% used both of these drugs, which was significantly more in comparison with women who were not exposed to violence ($p < 0.001$), while the use of alcohol was slightly higher among women who were exposed to violence ($p = 0.057$). Further, abortions over the previous year, were more reported by women exposed to violence (6.5% vs. 1.7%; $p = 0.001$).

Table 1

Sociodemographic characteristics of women exposed to domestic violence in the last 12 months

Sociodemographic characteristics	Total number	Abused women, n (%)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	*p*	OR (95% CI)	*p*
Age group (years)						
20–34	1,367	61 (4.5)	1.00		1.00	
35–49	1,590	86 (5.4)	1.25 (0.90–1.74)	0.183	1.02 (0.71–1.45)	0.932
50–75	2,931	160 (5.5)	1.28 (0.95–1.73)	0.099	0.95 (0.67–1.34)	0.767
Type of settlement						
urban	3,407	170 (5.0)	1.00		1.00	
rural	2,481	137 (5.5)	1.13 (0.89–1.43)	0.318	0.87 (0.65–1.15)	0.323
Marital status						
never married/never lived with a partner	779	24 (3.1)	1.00		1.00	
married/ living with a partner	3,942	214 (5.4)	1.92 (1.26–2.94)	0.002	1.90 (1.19–3.04)	0.007
widowed	804	36 (4.5)	1.64 (0.96–2.78)	0.069	1.39 (0.76–2.53)	0.288
divorced/separated	363	33 (9.1)	3.30 (1.93–5.66)	0.001	2.97 (1.66–5.31)	0.001
Education						
university degree	1,047	43 (4.1)	1.00		1.00	
secondary school	3,147	163 (5.2)	1.33 (0.94–1.87)	0.107	1.02 (0.65–1.58)	0.942
primary school	1,694	101 (6.0)	1.58 (1.09–2.28)	0.016	1.10 (0.77–1.59)	0.589
Wealth index						
rich	2,286	99 (4.3)	1.00		1.00	
middle	1,221	47 (3.8)	0.90 (0.63–1.29)	0.582	0.88 (0.61–1.28)	0.515
poor	2,381	161 (6.8)	1.64 (1.27–2.13)	0.001	1.58 (1.13–2.19)	0.007
Employment status						
employed	1,805	75 (4.2)	1.00		1.00	
unemployed/inactive	4,083	232 (5.7)	1.39 (1.06–1.81)	0.016	1.29 (0.96–1.75)	0.091
Social support (OSS-3)						
strong support	2,029	81 (4.0)	1.00		1.00	
moderate support	3,282	165 (5.0)	1.27 (0.96–1.67)	0.092	1.24 (0.94–1.63)	0.131
poor support	577	61 (10.6)	2.83 (1.98–4.03)	0.001	2.54 (1.77–3.64)	0.001

OR – odds ratio; CI – confidence interval.
OSS-3 – Oslo-3 Social Support Scale.

Table 2

Health consequences of ever-partnered women according to exposure to domestic violence during the last 12 months

Health indicators/risk behaviors	Exposure to domestic violence		
	yes n (%)	no n (%)	*p*
Self-perceived health			
good	106 (39.1)	2,231 (48.7)	0.001
fair	96 (35.4)	1,577 (34.5)	
poor	69 (25.5)	768 (16.8)	
Pain			
no pain/mild pain	108 (39.7)	2,401 (52.4)	0.001
moderate pain	79 (29.0)	1,429 (31.2)	
severe/very severe pain	85 (31.3)	750 (16.4)	
Filing tense or stressed/under pressure			
no	45 (16.6)	1,639 (35.8)	
yes	226 (83.4)	2,937 (64.2)	
Depressive disorder			
no depressive symptoms	172 (64.7)	3,867 (85.0)	0.001
mild depressive symptoms	62 (23.3)	498 (10.9)	
depression	32 (12.0)	188 (4.1)	
Sleeping pills/sedative use			
no	135 (49.7)	3,123 (68.2)	0.001
uses sleeping pills or sedatives	98 (36.0)	1,051 (22.9)	
uses both types of pills	39 (14.3)	406 (8.9)	
Alcohol consumption			
non-drinkers	157 (60.1)	2,845 (65.1)	0.057
moderate	79 (30.3)	1,260 (28.8)	
frequent	25 (9.6)	268 (6.1)	
Abortion			
no	145 (93.5)	2,693 (98.3)	0.001
yes	10 (6.5)	46 (1.7)	

Table 3

Association between exposure to domestic violence and health consequences among ever-partnered women in the last 12 months

Health indicators/risk behaviors*	OR		AOR†	
	OR (95% CI)	*p*	AOR (95% CI)	*p*
Self-perceived health as good	0.53 (0.38–0.72)	0.001	0.47 (0.32–0.71)	0.001
Presence of severe/very severe pain	2.45 (1.80–3.32)	0.001	2.41 (1.74–3.33)	0.001
Filing tense or stressed/under pressure	2.79 (2.02–3.86)	0.001	2.62 (1.89–3.64)	0.001
Depression (PHQ ≥ 10)	3.78 (2.52–5.68)	0.001	3.24 (2.08–5.03)	0.001
Use of sleeping pills or sedative	2.14 (1.64–2.81)	0.001	2.21 (1.67–2.93)	0.001
Frequent alcohol consumption	1.23 (0.96–1.59)	0.106	1.42 (1.08–1.86)	0.012
Abortion	4.04 (2.00–8.17)	0.001	3.11 (1.48–6.54)	0.003

***Dependent variables: self-perceived health as good vs. poor, presence of severe and very severe pain vs. no pain, filing tense or stressed/under pressure - yes vs. no, depression vs. no depressive symptoms, frequent alcohol consumption vs. non-drinkers, use of sleeping pills or sedative - yes vs. no, and abortion - yes vs. no; †Adjusted for age, type of settlement, marital status, education, wealth index, employment status and social support.**

OR – odds ratio; CI –confidence interval; AOR – adjusted odds ratio; PHQ – Patient Health Questionnaire.

Exposure to domestic violence during the last 12 months among ever-partnered women was significantly associated with a number of adverse health outcomes and risk behaviors (Table 3). Women who had experienced domestic violence were less likely to perceive their health as good than women who had not experienced domestic violence [adjusted OR (AOR) = 0.47; 95% CI = 0.32–0.71], and were more likely to report severe/very severe pain (AOR = 2.41; 95% CI = 1.74–3.33), stress and pressure exposure (AOR = 2.62; 95% CI = 1.89–3.64) and depression (AOR = 3.24; 95% CI = 2.08–5.03). Exposure to violence was also significantly associated with the use of sleeping pills or sedatives (AOR = 2.21; 95% CI = 1.67–2.93) and with the frequent use of alcohol (AOR = 1.42; 95% CI = 1.08–1.86). Women who were exposed to violence were more likely to have an abortion compared to those who were not exposed to violence (AOR = 3.11; 95% CI = 1.48–6.54).

Discussion

Our study showed that 4.9% of women aged 20–75 were exposed to physical and/or psychological violence in the family in the last 12 months (1.4% physical and 4.4% psychological violence). According to the results of the 2006 National Health Survey in Serbia, the percentage of exposure to physical violence in the last 12 months was 1.28%, which is similar to our result [20]. Unfortunately, the data are not completely comparable due to the different age limit of women, and also psychological violence was not included in that study.

The WHO Multi-Country Study on Women's Health and Domestic Violence (WHO-VAW Study) documented the widespread nature of IPV, with lifetime prevalence of physical and/or sexual partner violence among women ranging from 15% in Japan to 71% in Ethiopia. In most countries, between 15% and 30% (total range 4%–54%) of the women reported this violence within 12 months prior to the study [10]. According to the results of WHO-VAW Study conducted in Belgrade, 23.7% of women aged 15–49, experienced physical and/or sexual IPV at least once in their lifetime and 3.7% of women have experienced it in the last 12 months [13].

In Europe (European Union Member States), one in five women (22%) has ever experienced physical and/or sexual IPV and 4% have experienced it in the past year. The rates of partner violence for lifetime prevalence range from 30%–32% in Finland, Denmark and Latvia to 13% in Austria, Croatia, Poland, Slovenia and Spain. The experience of physical and/or sexual partner violence in the past 12 months range from 6% in Belgium, Bulgaria, Greece, Hungary, Italy, Romania and Slovakia, to some 2% in Estonia, Poland, Slovenia and Spain [21].

There are many studies related to domestic violence and their number is constantly increasing [5]. Unfortunately, comparison of results is difficult between different countries and the range in these figures illustrates not only possible real differences in prevalence rates among settings, but also differences in definitions of violence, study methodologies, as well as cultural differences pertaining to respondents' willingness to disclose acts of violence [6, 11, 22].

Studies find that psychological violence is more prevalent than physical or sexual IPV and that it also has significant health consequences [23, 24]. However, it is more rarely assessed in quantitative studies than physical and sexual IPV, and its definitions vary considerably [4, 12, 20]. Surveys indicate that physical violence in intimate relationships is almost always accompanied by psychological abuse. In addition, psychological violence by partners is highly correlated with physical violence and is an important long-term predictor of physical violence at the early stages of marriage [9]. Results of the study "Mapping family violence against women" from 2010, which was conducted on a representative sample of 2,500 women aged 18–75 in Serbia, showed that the most frequent form of violence in last 12 months is psychological (31.8%), followed by physical (10.1%) and economical (11.4%), while the less frequent is sexual violence (1.2%) [9].

According to the results of this study, exposure to domestic violence was not significantly associated with the age of women. The same results were obtained in the 2006 National Health Survey [20]. However, the WHO-VAW Study and many other researches showed that in almost all parts of the

world partner violence occurs in younger women and this pattern may reflect that physical violence mostly appears at an early stage of a partner relationship [10, 24, 25]. Consistent with other studies, our results indicate that divorced or separated women in comparison with married ones have a greater chance of being exposed to violence by a partner [8, 24, 26]. These results suggest that there is a possibility that married women are underreporting domestic violence, and that divorced/separated women are most probably more willing to disclose domestic violence than married women. Conclusively, domestic violence can be an important reason for marriage dissolution [8, 24, 27]. In our results, exposure to violence among women showed an association with material status and social support, while association with education and employment status was significant only in univariate regression analysis. On the other hand, numerous previous researches suggest that education has a protective effect for women on IPV risk [25–29]. Women with higher education probably have a greater opportunity of choice in partners and more ability to decide whether to marry or not, as well as to negotiate greater autonomy and control of resources within the marriage [10]. Employment status and financial autonomy are also probably protective factors against IPV exposure [10, 15, 30]. In developed countries, economic independence protects women and allows them to leave a violent partner [3, 14, 25, 27]. In numerous studies, higher socioeconomic status is generally associated with lower levels of physical and/or sexual partner violence [20, 26, 31]. On the other hand, some researches showed that the wealth of a household has an inconsistent and often nonlinear relationship with the experience of violence [3, 8].

The findings that women with poor social support have a greater chance of exposure to violence have been confirmed in other studies [24, 29]. Studies confirmed that victims' social contacts were controlled by their partners [12]. The WHO defined the following behavior by a woman's partner as: restricting contact with her family of birth and friends; controlling her access to health care; accusing her of being unfaithful etc. The proportion of women reporting one or more of these behaviors by their partner varied from 21% in Japan to almost 90% in the United Republic of Tanzania [10].

Authors also include other characteristics of women who are associated with partner violence such as: sexually abused as a child, unwanted first sexual intercourse, poor self-esteem, having a mother who was beaten by her partner, etc. [24, 26, 28, 29]. On the other hand, studies also indicate that the majority of factors associated with IPV against women are factors related to the male partner (alcohol consumption, being less educated, infidelity, his personal experiences of violence in childhood, aggressive behaviour towards other men, his mother was abused by mother's partner, etc.) [23, 24, 28, 29].

In our study, significant associations of exposure to domestic violence among ever-partnered women with self-perceived poor health, as well as specific health problems and risk behaviors: severe or very severe body pain, stress and pressure, depression, use of sedative or sleeping drugs, frequent use of alcohol and abortion were found. This is consistent with the findings of the current studies which showed that experience of physical or psychological IPV was significantly associated with self-reported poor health and a range of adverse physical, mental, sexual and reproductive health outcomes [10, 12, 23, 26, 32].

Health consequences of domestic violence against women were well documented. Compared with women who never experienced IPV, women who experienced IPV were more likely to report pain (whole-body pain, chronic neck or back pain, chest pain, headache, migraines, abdominal pain, pelvic pain), difficulties with walking or daily activities, an increase in disability days and overall disability, memory loss, dizziness, problems seeing even with glasses [8, 11, 12, 20, 23, 32, 33], injuries, gastrointestinal disorders (spastic colon, frequent ingestion, constipation or diarrhoea), high blood cholesterol, heart disease, heart attack, arthritis, stroke, asthma [12, 23, 31–33], gynecological problems, vaginal discharge, foot oedema, eczema [8, 10, 11, 20, 31].

Some investigations showed that women who had experienced partner violence had an increased risk of mental health disorder, anxiety, mood disorder, posttraumatic stress disorder (PTSD), beginning to stammer or stutter, insomnia and chronic mental illnesses including depression [22, 23, 30, 32–36]. Studies also showed that women who had experienced IPV (physical, sexual or both), were significantly more likely to have suicidal ideas or attempted suicide than nonabused women [11, 32, 34].

IPV has been associated also with health risk behaviors: smoking, marijuana use, heavy or binge drinking, risk factors for human immunodeficiency virus (HIV) or sexually transmitted diseases [31, 36]. There is plentiful evidence of the association between alcohol use and domestic violence, particularly around IPV perpetration by men [23, 28, 29, 36, 37]. There is also clear evidence that women with histories of violence consume more alcohol [31, 35, 37]. However, the causal direction of the linkage between alcohol consumption by women and their experiences of IPV is less clear. Alcohol use can be both a cause and a consequence of experiencing violence [37]. The WHO-VAW Study showed that in all the investigated sites odds of IPV were higher in relationships where one or both partners had problems with alcohol [26]. In Serbia, frequent use of alcohol was reported by 11% of women who have experienced physical and/or sexual violence. Alcohol abuse by partners in Serbia was at third place on the list of reasons for violence with 23.8% (at first place – without any reason 28.2% and at second place – jealousy with 24.2%) [13]. Our findings are in accordance with the majority of studies showing association between the exposure to violence and tranquilizer/sedative use, antidepressant use and prescription pain pill use [12, 32–34]. The study in Belgrade also showed that women reporting physical violence and/or sexual violence committed by partners were more often using sleeping medication (11.3%), pain killers (21.3%) or antidepressants (3.9%) compared to women not reporting violence [13].

Different types of injuries could be the result of a violent event and, directly or indirectly, endanger a woman's life or cause fatal outcome [1, 4]. Under the WHO-VAW Study, in seven out of ten countries participating, over 15% of ever-injured women reported that injury had happened more than five times ever in her life [11]. In Belgrade, injuries as a result

of violence were reported by 28.8% of women and, among them, every third one (35.8%) has been injured more than five times [13].

IPV is a strong risk factor for unintended pregnancy and abortion across a variety of settings. Our results showed that women exposed to violence had a more than three times higher chance of an abortion compared to women without such an experience, which is consistent with other studies [10, 22, 33]. Within the study conducted in Belgrade, 20.7% of women exposed to physical and/or sexual violence had a spontaneous miscarriage and 65.0% had an abortion (compared to 45.6% of women not experiencing violence) [13].

The violence is often hidden within family and partner relations, and is quite difficult to be documented. In Serbia, 78.2% of women exposed to violence never asked for help from competent institutions regarding physical and/or sexual violence. Women who experienced violence often deny such experience due to fear of the perpetrator [4, 13]. Strict application of legal provisions regulating this field [38], as well as proper training of the police, the judicial sector, social and health care providers are a necessary precondition to early detection of domestic violence, as well as adequate and due help to victims. The role of a healthcare professional is particularly relevant and important in addressing domestic violence. Health providers, in most cases, do not consider domestic violence as part of their competencies. Therefore, it is necessary to be trained to recognize violence, react adequately as well as document and report violence. Further implementation of the Special Protocol for the Protection and Treatment of Women Victims of Violence from the Ministry of Health of the Republic of Serbia is particularly important in order to protect women from violence [4].

Limitation of the study

We used data from the National Health Survey which may not have been sensitive for measuring domestic violence. Even though the self-administered questionnaire was used, it is likely that exposure to domestic violence among women was underreported in this study (6.8% of women did not answer the question related to the exposure to domestic violence). Finally, as the study was cross-sectional, we are not able to draw any conclusions about cause and effect. Despite this limitation, using data from the National Health Survey we were able to assess a larger context in which violence takes place, and also the association between domestic violence and health. A special advantage is that the results are based on data from a nationally representative population sample, which provides reliable statistical analysis.

Conclusion

This study was shown the association of domestic violence against women with adverse health outcomes. Violence prevention demands a wide public health response and the health sector has a central role. The practical implications of our findings are relevant to physicians. Results indicate that special attention should be paid by physicians in recognising all symptoms, physical and mental health disorders that might indicate exposure to violence as well as documenting violence. This study also offers important results that can be especially useful to policy-makers.

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Peri-implant soft and hard tissue condition after alveolar ridge preservation with beta-tricalcium phosphate/type I collagen in the maxillary esthetic zone: a 1-year follow-up study

Stanje tvrdog i mekog periimplantnog tkiva u estetskoj regiji gornje vilice posle prezervacije alveolarnog grebena beta-trikalcijum fosfatom sa kolagenom tip I: Studija sa jednogodišnjim periodom praćenja

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Abstract

Background/Aim. Alveolar ridge dimensional alterations following tooth extraction in the anterior maxilla often result in an inadequate bone volume for a correct implant placement. In order to obtain optimal bone volume various bone graft substitutes have become commercially available and widely used for socket grafting. The aim of this study was to examine and compare long-term clinical outcomes of dental implant therapy in the maxillary esthetic zone, after socket grafting with beta-tricalcium phosphate (TCP) combined with collagen type I, either with or without barrier membrane and flap surgery, after a 12-month follow-up. **Methods.** Twenty healthy patients were allocated to either C group (beta-TCP and type I collagen without mucoperiosteal flap coverage) or C+M group (beta-TCP and type I collagen barrier membrane with mucoperiosteal flap coverage). Following clinical parameters were assessed: implant stability (evaluated by a resonance frequency analysis – RFA), periimplant soft tissue stability (sulcus bleeding index – SBI, Mombelli sulcus bleeding index – MBI, periimplant sulcus depth, keratinized gingiva width, gingival level) and marginal bone level at the retroalveolar radiograms. **Results.** Within C+M group, RFA values significantly increased 12 weeks after implant installation compared to primary RFA values. Comparison between investigated groups showed a significantly reduced keratinized gingiva width in the C+M group compared to the C group after 3, 6, 9 and 12 months. Comparison between groups revealed significantly lower gingival level values in the C+M group at 9th and 12th month when compared to the C group. **Conclusion.** Implant treatment in the anterior maxilla could be effective when using a 9 months alveolar ridge preservation healing with combined treatment with beta-tricalcium phosphate and type I collagen, with regard to the peri-implant soft and hard tissue stability.

Key words:
dental implants; tooth extraction; bone substitutes; calcium phosphates; collagen; maxilla.

Apstrakt

Uvod/Cilj. Posle ekstrakcije zuba, dimenzionalne promene alveolarnog grebena u estetskoj regiji gornje vilice za posledicu često imaju nedovoljnu količinu kosti za ugradnju zubnih implanata. U vezi sa tim, primenjuju se različiti koštani zamenici sa ciljem očuvanja dimenzija alveolarnog grebena posle ekstrakcije zuba. Cilj rada bio je da se, posle prezervacije alveolarnog grebena beta-trikalcijum fosfatom (TCP) sa kolagenom tip 1, sa barijernom membranom i mukoperiostalnim režnjem i bez nje, ispitaju i uporede klinički ishodi zarastanja posle ugradnje zubnih implanata u estetskoj regiji gornje vilice, tokom jednogodišnjeg perioda praćenja. **Metode.** Dvadeset zdravih bolesnika podeljeno je u dve grupe: C (beta TCP/kolagen tip 1 bez barijerne membrane i mukoperiostalnog režnja) i C+M (beta TCP/kolagen tip 1 sa barijernom membranom i mukoperiostalnim režnjem). Praćeni su uobičajeni klinički parametri ishoda terapije: implantna stabilnost (analiza rezonantne frekvence), stanje mekih tkiva (indeks krvarenja, plak indeks, širina pripojne mukoze, recesija gingive) i nivo periimplantnog koštanog tkiva na retroalveolarnom radiogramu. **Rezultati.** U C+M grupi, implantna stabilnost posle 12 nedelja bila je značajno veća u odnosu na primarnu stabilnost. U C+M grupi, širina keratinizovane gingive bila je značajno manja posle 3, 6, 9 i 12 meseci u odnosu na C grupu. Recesija gingive bila je

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značajno veća u C+M grupi u odnosu na C grupu posle 9 i 12 meseci. **Zaključak.** Razmatrajući stabilnost mekog i tvrdog periimplantnog tkiva, terapija zubnim implantima može biti uspešna prilikom ugradnje u estetskoj regiji gornje vilice.

Ključne reči:
implanti, stomatološki; zub, ekstrakcija; kost, zamenici; kalcijum fosfati; kolagen; maksila.

Introduction

Single tooth replacement with an implant-supported restoration has become a viable treatment option in the maxillary esthetic region. However, alveolar ridge alterations after tooth extraction in the anterior maxilla often result in an inadequate bone volume. Buccal bone plate is usually resorbed during the first 8 weeks after tooth removal, leading to a predominantly horizontal alveolar ridge reduction in the following year [1–3]. In the systematic review, Tan et al. [4] reported the alveolar ridge reduction of 3.8 mm in width and 1.2 mm in height in the first 6 months after tooth removal. Mucosal changes after tooth extraction, consist of gaining thickness at the alveolar ridge crest, which increases by 0.4 mm after 4 months of healing. However, reduced bone volume, both vertically and horizontally, follows changes in the underlying alveolar bone [5, 6]. Although successful osseointegration of dental implants is highly predictable nowadays, a long-term outcome has been evaluated in view of the esthetic and functional stability. Taking into account long-term clinical results, it is well known that sufficient facial bone thickness is required to allow peri-implant soft and hard tissue stability and favorable esthetic outcome [7, 8].

To obtain an adequate bone volume after tooth extraction, different adjunctive procedures (alveolar ridge preservation, socket grafting, immediate implant placement) and different biomaterials (autografts, xenografts, synthetic biomaterials) have been proposed, resulting in less vertical and horizontal alveolar ridge alterations, which might prevent extensive bone augmentation techniques at later stages [9–14]. Despite the fact that autogenous bone grafts are considered as a *gold standard* due to viable bone cells and osteogenic potential, several limitations such as the presence of additional surgical site and morbidity, unpredictable graft resorption and limited bone volume may be disadvantages of this procedure [15–19]. Therefore, in order to obtain optimal bone volume in a minimally invasive manner, various bone graft substitutes have become commercially available and widely used for the alveolar ridge preservation. Bone graft substitutes may be used either alone or in combination with autogenous bone particles, and with or without barrier membrane coverage [14, 20, 21]. The use of barrier membranes prevents growing of fast proliferating fibrous tissue into a bony defect, which allows undisturbed bone regeneration, with fast clot formation and wound stabilization [22]. However, it has to be noted that exposure, infection or disintegration of the barrier membrane may lead to a failure of the grafting procedure [23]. Also, to obtain full barrier membrane coverage, esthetic outcome may be affected by mucoperiosteal flap elevation due to a reduction of keratinized gingiva in the grafted region. Data from experimental studies showed that the bone remodeling after tooth extraction is less pronounced after alveolar ridge preservation with flapless procedure [7]. On the other hand, in the study of Barone et al. [24], no histological and histomorphometric differences were observed 3 months after socket grafting with cortico-cancellous porcine bone covered with resorbable barrier membrane, comparing flapless and flap elevation procedures.

Beta-tricalcium phosphate (beta-TCP) is a bioactive bone substitute material with an osteoconductive and favorable resorptive properties [25], and the ability to support formation of new bone in grafted areas [26–28]. These properties were demonstrated even when beta-TCP was used without barrier membrane for grafting procedures during maxillary sinus floor augmentation or cyst removal in the mandible [29]. Beta-TCP may be successfully combined with collagen [30], although it was demonstrated that collagen alone is not capable of improving bone remodeling and counteracting post-extraction alveolar ridge alterations [31, 32]. Histologic, histomorphometric and immunohistochemical analyses showed that beta-TCP with type I collagen, either with or without barrier membrane and mucoperiosteal flap coverage, produced sufficient amounts of vital bone for consequent implant installation, with similar potential for bone healing during a 9-month observation period [20].

To our knowledge, there are no data reporting benefits of alveolar ridge preservation procedure on the long-term outcomes of implant treatment in the maxillary esthetic zone. Therefore, the aim of this study was to examine and compare long-term clinical results concerning quality of peri-implant tissue in the maxillary esthetic zone after alveolar ridge preservation with beta-TCP combined with type I collagen, either with or without barrier membrane and flap surgery.

Methods

Study sample and design

Ethics approval was obtained from the Ethics Committee of the Faculty of Dental Medicine, University of Belgrade (No. 36/21) and all participants signed the informed written consent. Study registration was performed at ClinicalTrials.gov (NCT02507661) and study has been conducted in accordance with the ethical standards laid down in 1964 Declaration of Helsinki and its later amendments. This randomized study included 20 adult participants of both genders, aged between 18 and 65 years, referred to the Oral Surgery Clinic for single maxillary tooth extraction and post-extraction alveolar ridge preservation, prior to dental implant placement.

Inclusion criteria were: healthy patients (ASA I physical status) with single maxillary tooth in the maxillary es-

thetic zone (incisors, canines or premolars) indicated for extraction due to a root fracture, unsuccessful endodontic treatment or chronic periodontal disease, and with at least 6 mm of remaining alveolar height; extraction sockets with four intact bony walls and thick, medium and thin gingival biotype; adequate occlusion for the proposed prosthodontic treatment. Patients were excluded in cases of: heavy smoking, acute periodontal disease with severe bone loss, chronic orofacial pain, pregnancy and lactation, and alcohol and/or drug abuse.

Study procedure

All extractions were performed under local maxillary infiltration anesthesia (2 mL of 4% articaine with epinephrine 1:100.000) in a minimally traumatic manner. After a tooth extraction, an alveolar socket debridement was done and single beta-TCP cone with type I collagen (RTR Cone®, Septodont, France) was placed into the socket to completely fill the space. Participants were randomly assigned to one of the following two groups: group C (beta-TCP + type I collagen) – 11 participants with cones placed into the extraction socket without barrier membrane and mucoperiosteal flap coverage; group C+M (beta-TCP + type I collagen with membrane) – 9 participants with cones placed into the extraction socket and covered with barrier membrane (Bio-Gide®, Geistlich AG, Switzerland) and mucoperiosteal flap.

In the C+M group, full thickness mucoperiosteal flap was elevated, following two vertical and horizontal intrasulcular incisions. Periosteal incision was performed to obtain necessary flap mobility for the cone and barrier membrane complete coverage, followed by interrupted sutures.

Postoperatively, participants were instructed to take amoxicillin (Sinacilin® 500 mg, Galenika, Serbia), 3 times daily for 7 days and ibuprofen (Brufen® 400 mg, Galenika, Serbia) as necessary, as well as to follow the postoperative protocol (antiseptic mouth wash twice daily for ten days and soft diet). Participants attended regular check-ups at 3rd, 5th and 7th day. Sutures were removed after 7 days.

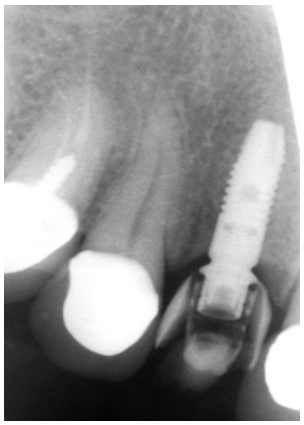

Fig. 1 – Periapical radiograph with screw-retained temporary crown.

Dental implants (AstraTechOsseoSpeed TX®, Dentsply Implants, Sweden) were installed 9 months after the socket preservation according to the delayed implant placement protocol, followed by temporary crown for first 2 months (Figure 1) and screw-retained final metalo-ceramic crown delivery (after 2 months of temporary crown).

Clinical parameters

Clinical parameters evaluated during the follow-up period were: implant stability, peri-implant soft tissue stability and peri-implant bone level changes.

Implant stability was evaluated by means of resonance frequency analysis (RFA) using OstellMentor® appliance (Integration Diagnostics, Sweden). The transducer from the appliance set was perpendicularly positioned into the implant body (Figure 2) and measurements were repeated until two identical RFA values were obtained, which was considered as a value of implant stability. Measurements were performed immediately after implant placement and after 3, 6, 8 and 12 weeks postoperatively.

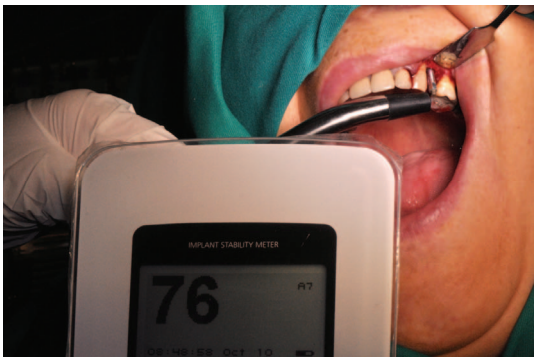

Fig. 2 – Implant stability measurement with OstellMentor® appliance.

Peri-implant soft tissue stability was assessed according to a Mombelli sulcus bleeding index (SBI), Mombelli modified plaque index (MPI) and with following gingival parameters: peri-implant sulcus depth, keratinized mucosa width and gingival level. SBI and MBI were measured at the mesial, distal, buccal and palatal aspect of each implant [33]. Peri-implant sulcus depth was evaluated at the same four sites *per* implant. Measurements were performed at the midfacial aspect of the implant as the distance between the most coronal gingival margin and the sulcular depth. Keratinized gingiva width was measured at the midfacial aspect of the implant as the distance between midfacial gingival margin and mucogingival junction. Gingival level was measured at the midfacial position of buccal mucosa as the distance of marginal gingiva and mucogingival junction, registering the level of gingival recession. Measurements were performed 2, 3, 6, 9 and 12 months after the implant placement using manual periodontal probe.

Peri-implant bone level changes were measured on periapical radiographs, taken with parallel technique immediately after implant placement (Figure 3), as well as after 3, 6, 9 and 12 months. The marginal bone level was regarded as the distance between the implant-abutment connection and the first bone-to-implant contact. All measurements were performed at the mesial and distal aspects of each implant in the specialized image software (ImageJ, National Institute of Health, USA).

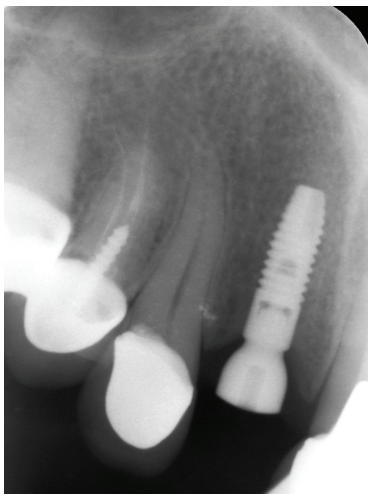

Fig. 3 – Periapical radiograph immediately after implant placement.

Statistical analysis

Statistical analysis was performed in SPSS v.20. Demographic data were analyzed by means of descriptive statistics, χ^2 and Man Whitney *U* test. Clinical parameters were compared between groups using Mann Whitney *U* test, while the changes within investigated groups during follow-up period were analyzed by Friedman test with Wilcoxon Sign Rank post hoc. The level of statistical significance was set at 0.05.

Results

Characteristics of the study population are presented in Table 1. There were no statistically significant differences between the investigated groups regarding age, smoking habits, dental diagnosis as well as implant distribution according to dimensions.

Implant stability analysis revealed that there were no significant differences in RFA values within the C group, during the observation period. Within the C+M group, RFA values significantly increased 12 weeks after implant installation in comparison with primary stability values (Table 2). Comparison between investigated groups did not show significant differences in RFA values during the observation period (Table 2).

Table 1
Demographic and surgical data of the study population

Parameters	Group C	Group C+M
Patients, n	11	9
Age (years), mean ± SD	49 ± 15	46 ± 13
M/F (n)	5/6	3/6
Smoker/non smoker, n	4/7	5/6
Diagnosis, n		
A/B/C/D	2/6/2/1	2/3/1/3
Implants, n		
$3.5^a \times 11^b$	5	5
$4.0^a \times 11^b$	6	4

Group C – beta-tricalcium phosphate (TCP) and type I collagen without mucoperiosteal flap coverage;

Group C+M – beta-TCP and type I collagen barrier membrane with mucoperiosteal flap coveage;
M – males; F – females; A – periodontal disease; B – non-vital tooth; C – chronic periapical lesion; D – tooth fracture; n – number of patients; SD – standard deviation
[a] –implant diameter in mm; [b] – implant lenght in mm.

Table 2
Resonance fraquency analysis values during the observation period

Weeks	Group C* (mean ± SD)	Group C+M* (mean ± SD)	p^a
0	69.6 ± 6.2	69.4 ± 5.9	n.s.
3	66.4 ± 4.9	66.6 ± 5.7	n.s.
6	68.1 ± 4.9	71.3 ± 4.6	n.s.
8	70.5 ± 5.2	73.9 ± 4.5	n.s.
12	74.3 ± 6.4	76.4 ± 5.4*	n.s.
p^b	0.11	0.01	

***Explanation see under Table 1.**
SD – standard deviation; [a]Mann-Whitney test; [b]Friedman test; [*] $p < 0.05$ – 0 vs. 12th week (Wilcoxon Sign Rank *post hoc*).

Values of bleeding and plaque indices did not change significantly during the observation period except between the C and C+M groups concerning the Mombelli plaque index, 3 months after implant placement (Table 3).

Keratinized gingiva width was not significantly changed within investigated groups during the 12-month period of observation (Table 4). However, comparison between the investigated groups showed a significantly reduced keratinized gingiva width in the C+M group starting from the 3rd month, compared to the C group (Table 4).

Table 3
Values of bleeding and plaque indices (Mombelli) during the observation period

Month	Bleeding index			Plaque index		
	Group C* (mean ± SD)	Group C+M* (mean ± SD)	p^a	Group C (mean ± SD)	Group C+M (mean ± SD)	p^a
2	0.10 ± 0.31	0.13 ± 0.35	n.s.	0.20 ± 0.63	0.38 ± 0.74	n.s.
3	0.40 ± 0.52	0.48 ± 0.52	n.s.	0.25 ± 0.53	0.50 ± 0.46	< 0.05
6	0.20 ± 0.32	0.33 ± 0.54	n.s.	0.30 ± 0.68	0.50 ± 0.76	n.s.
9	0.60 ± 0.52	0.63 ± 0.52	n.s.	0.20 ± 0.42	0.38 ± 0.52	n.s.
12	0.40 ± 0.52	0.25 ± 0.36	n.s.	0.20 ± 0.42	0.25 ± 0.46	n.s.
p^b	n.s.	n.s.	n.s.	n.s.	n.s.	

***Explanation see under Table 1.**
SD – standard deviation; [a]Mann-Whitney test; [b]Friedman test; Wilcoxon Sign Rank *post hoc*.

Table 4

Peri-implant soft tissue parameters during the observation period

Months	Keratinized gingiva			Peri-implant sulcus depth			Gingival level		
	Group C[1]	Group C+M[1]	p^a	Group C	Group C+M	p^a	Group C	Group C+M	p^a
2	3.6 ± 1.0	3.0 ± 1.1	n.s.	2.40 ± 0.71	1.80 ± 0.63	n.s.	2.37 ± 0.42	1.98 ± 0.68	n.s.
3	3.8 ± 0.9	2.9 ± 0.7	0.047	2.40 ± 0.84	2.00 ± 1.11	n.s.	2.41 ± 0.42	1.95 ± 0.57	n.s.
6	3.9 ± 1.0	2.8 ± 0.8	0.035	2.31 ± 0.97	2.20 ± 1.07	n.s.	2.33 ± 0.70	1.76 ± 0.41	n.s.
9	3.7 ± 0.9	2.7 ± 0.8	0.013	2.88 ± 0.68	2.29 ± 0.35	0.03	1.88 ± 0.66	1.29 ± 0.35	0.04
12	3.7 ± 0.9	2.7 ± 0.9	0.011	2.85 ± 0.65	2.20 ± 0.21	0.04	1.86 ± 0.69	1.18 ± 0.21	0.04
p^b	n.s.	n.s.		0.032	0.048		n.s.	0.035	

Values given as mean ± standard deviation in mm.
[1]Explanation see under Table 1.
[a]Mann-Whitney test; [b]Friedman test; Wilcoxon Sign Rank *post hoc*.

Comparing peri-implant sulcus depth within C and C+M groups, there was a significant increase of sulcus depth after 12 months in comparison with the 2nd month (Table 4). Significant differences regarding this parameter between investigated groups were also obtained after 9 and 12 months (Table 4).

Gingival level was significantly reduced in the C+M group after 9 and 12 months of observation (Table 4). There were no significant differences in gingival level in the C group. Between groups comparison revealed significantly lower gingival level values in the C+M group at the 9th and 12th month when compared to the C group (Table 4).

Peri-implant bone levels did not change significantly during a 12-month observation period, neither within nor between the investigated groups (Table 5).

Table 5

Radiographic evaluation of the peri-implant bone level

Months	Group C* (mm) mean ± SD		Group C+M* (mm) mean ± SD		p^a
	mesial	distal	mesial	distal	
2	0.7 ± 0.7	0.6 ± 1.2	0.8 ± 0.7	0.9 ± 0.9	n.s.
6	0.9 ± 0.7	1.2 ± 1.1	1.3 ± 0.9	1.4 ± 1.1	n.s.
9	1.0 ± 0.5	1.2 ± 1.0	1.1 ± 0.6	1.1 ± 0.4	n.s.
12	1.3 ± 0.7	1.6 ± 1.0	1.4 ± 0.6	1.8 ± 0.2	n.s.
p^b	n.s.	n.s.	n.s.	n.s.	

***Explanation see under Table 1.**
[a]Mann-Whitney test (comparison between groups for mesial and distal side);
[b]Friedman test, Wilcoxon Sign Rank *post hoc*.

Discussion

RFA values obtained in our study imply high levels of primary and secondary implant stability in both investigated groups for 12 weeks observation period (> 65 implant stability quotient – ISQ). It should be noticed that implants were placed in the solid, mostly mineralized alveolar bone, 9 months after preservation, where implant micro-movements, evident after immediate placement, were not present. Expected decrease in implant stability was observed after 3 weeks in both groups because of bone healing and remodeling processes, but transition from primary stability as a mechanical phenomenon to secondary stability as biological type of bone-to-implant connection [34] was evident. In the C+M group significant increase in RFA values (and implant stability) was observed at 12 weeks in comparison with primary stability values, while in the C group significant changes were not observed. This difference may be explained with a pattern of bone healing in non-membrane group, which is characterized by thin immature trabecular bone in cervical and central part of the post-extracting preserved socket [20].

Marginal bone remodeling occurred in both investigated groups, with similar values between groups at the mesial and distal implant sides during the observation period of 12 months. Slightly higher values of 1.9 mm were observed in the C+M group compared to 1.6 mm in the C group at the end of the observation period, but differences were not significant. The first progressive bone loss in our study occurred during first 6 months after the implant placement, 1.2 mm at the distal side in the C group and 1.4 mm at the distal side in the C+M group. These results are in agreement with the study of Cochran et al. [35], who reported that the most pronounced peri-implant bone remodeling occurs during first 6 months after one-stage protocol implant installation, although reported mean values in the study were 2.44 mm. This reduction is probably a result of early bone remodeling during the first year with implant osteotomy preparation, interruption of vascular supply and possible inflammation [35]. In the study of Hartman and Cochran [36], after using the same one stage protocol, the most bone loss also occurred during first 6 months after implant installation, with average bone loss of 1.10 mm. The authors concluded that the early bone loss directly depends on the implant design and three-dimensional implant position. Concerning that, it is explained that this process depends on various factors, including type of implant-abutment connection, as well as implant neck surface characteristics [37–40]. It seems that tapper connection of implants used in this study, with internal hexagon, allows horizontal displacement of implant-abutment interface. It is reported that this type of connection leads to the lesser apical migration of biological width, since micro-movements and stress transmission occur at a distance from the marginal bone, which is followed by less marginal bone resorption [41–43].

The important part of analysis was the peri-implant soft tissue stability. The midfacial soft tissue level (gingival level) significantly decreased in the C+M group after 9 and 12 months in comparison with the C group. Furthermore, the gingival recession in the C+M group at mentioned time

points was significantly lower in comparison with baseline measurement. The observed pattern of the midfacial soft tissue recession is possibly a result of restoring adequate biological dimensions of the tissue; it seems to be present during early healing phase irrespectively of implant treatment modality, especially when flap surgery was done. Similar values were obtained in studies with single-tooth implants installation with standard surgical approach [44], as well as after single-tooth implants installed with bone augmentation procedure [45].

Most clinical studies reported that the amount of gingival recession significantly increased at the implant sites with reduced keratinized mucosa [46–48]. This is in accordance with our results of keratinized mucosa level and gingival recession in the C+M group, pointing that the deficient keratinized mucosa is related with the increased gingival recession. Furthermore, buccal probing depth showed a tendency to be slightly higher in the sufficient keratinized mucosa, while plaque and bleeding index were higher when keratinized mucosa was deficient, what is in accordance with previously published data [46–48].

From a clinical point of view, stability of peri-implant crestal bone level is crucial for a long-time implant outcome in the maxillary esthetic zone. Namely, an appropriate amount of keratinized mucosa prevents mucosal traction during masticatory function, which is a positive influence of a wide keratinized mucosa of 2 mm on a crestal bone level. Regarding the proper width of keratinized mucosa, the better results of the C group could be explained with higher tissue stability and lower biofilm accumulation. Conversely, sites with deficient keratinized mucosa have a potential difficulty in maintaining adequate health of peri-implant tissue [49]. Additionally, keratinized mucosa in the vicinity of implants probably reduces inflammatory alterations of connective tissue, which is in accordance with other studies [50].

Conclusion

This clinical study showed that treatment of the maxillary esthetic zone could be effective using 9 months alveolar ridge preservation healing combination of beta-tricalcium phosphate and type I collagen in a term of the peri-implant soft and hard tissue stability. Marginal mucosa stability strongly affects the esthetic outcomes in the restored maxillary esthetic zone if gingival recession occurs. Further data on the long-term survival and success rates of dental implants are needed.

Acknowledgements

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Postmortem serotonin level in cerebrospinal fluid as a marker of the manner of death

Postmortalne vrednosti serotonina u cerebrospinalnoj tečnosti kao marker porekla smrti

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Abstract

Background/Aim. Serotonin [5-hydroxytriptanine (5-HT)], as a neurotransmitter in the central nervous system, is included in the regulation of autonomic and cognitive functions, sensory processing, motor activity, emotions, mood, and almost every kind of behavior. In forensic investigations of death, 5-HT has been studied in different body fluids, regarding the cause of death, particularly in suicides and drug abuse or as a marker of an acute stress response. The aim of this study was to establish 5-HT levels in cerebrospinal fluid (CSF) as a marker of its central activity during fatal event in deaths different in manner, particularly in cases where the victims were aware of the stressful event. **Methods.** Study sample consisted of 81 postmortem CSF obtained during autopsy. Concentrations of the 5-HT were established regarding natural versus violent (accidents, homicides and suicides) deaths. After preparation, samples were analyzed through the liquid chromatography-tandem mass spectrometry method. **Results.** Violent deaths had significantly higher 5-HT levels (U = 519.000; $p < 0.05$). Differences were found in mean values among different causes of death (higher in blunt injury, stabbing and intoxication, while lower in cardiac deaths and hypothermia) but without statistical significance. 5-HT levels significantly differed among age groups (χ^2 = 13.354; p = 0.001), with the tendency to decrease with age. No differences in 5-HT levels were observed regarding gender, length of agony period, and awareness of impending lethal outcome. The values tended to increase with postmortem interval albeit without significant differences. **Conclusion.** Serotonin could be a useful postmortem biochemical marker to distinguish natural and violent death, regardless of individual variability in concentrations.

Key words:
autopsy; biomarkers; cause of death; cerebrospinal fluid; chromatography, liquid; death; forensic medicine; serotonin.

Apstrakt

Uvod/Cilj. Serotonin [5-hidroksitriptamin (5-HT)], kao neurotransmiter u centralnom nervnom sistemu (CNS), uključen je u regulaciju autonomnih i kognitivnih funkcija, obrade senzornih informacija, motorne aktivnosti, emocija, raspoloženja i gotovo svakog oblika ponašanja. U sudskomedicinskoj istrazi, 5-HT je proučavan u različitim telesnim tečnostima u odnosu na uzrok smrti, a posebno u vezi sa samoubistvima, zloupotrebom droga ili kao marker akutnog odgovora na stres. Cilj ove studije bio je utvrđivanje nivoa 5-HT u cerebrospinalnoj tečnosti, kao markera njegove centralne aktivnosti tokom procesa umiranja, u smrtima različitog porekla, a posebno u slučajevima gde su žrtve bile svesne stresnog događaja. **Metode.** Istraživanje je sprovedeno na postmortalnim uzorcima 81 likvora uzetih tokom obdukcije. Koncentracije 5-HT su određivane u odnosu na prirodno i nasilno (zadesno, samoubilačko i ubilačko) poreklo smrti. Nakon pripreme, uzorci su analizirani metodom tečne hromatografije sa tandem masenom spektrometrijom. **Rezultati.** Vrednosti 5-HT su bile značajno više u slučajevima nasilnih smrti u odnosu na prirodne smrti (U = 519,000; $p < 0.05$). Utvrđene su razlike u srednjim vrednostima 5-HT između različitih uzroka smrti (usled trovanja, povređivanja dejstvom tupine, šiljka i oštrice, a niže u hipotermiji i srčanoj smrti). Vrednosti 5-HT značajno su se razlikovale među grupama (χ^2 = 13,354; p = 0.001, sa tendencijom sniženja sa godinama. Nisu utvrđene razlike u odnosu na pol, dužinu agonije i svesnost o nastupajućem smrtnom ishodu. Vrednosti su imale tendenciju rasta sa dužinom postmortalnog intervala, ali se to nije pokazalo statistički značajnim. **Zaključak**. Serotonin bi mogao biti koristan postmortalni biohemijski marker u razlikovanju prirodnih i nasilnih smrti, uprkos velikim individualnim varijacijama.

Ključne reči:
autopsija; biološki pokazatelji; smrt, uzrok; cerebrospinalna tečnost; hromatografija, tečna; smrt; medicina, sudska; serotonin.

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Introduction

Serotonin, a biogenic monoamine, chemically identified as 5-hydroxytryptamine (5-HT), acts both as a neurotransmitter in the central nervous system (CNS) and a local hormone in the peripheral tissues [1]. Through the projections, 5-HT is involved in the regulation of sleep, appetite, sexual activity, body temperature, circadian rhythms, autonomic and cognitive functions, sensory processing, motor activity, emotions, mood, i.e. almost every kind of behavior. In forensic investigation of death, 5-HT has been studied in different body fluids, regarding the cause of death – particularly suicides and drug abuse or as a marker of an acute stress response [2–7] with a significant contribution.

Briefly, stress is a biological response to real/potential threat to body integration and homeostasis, which initiates numerous autonomic, endocrine, cognitive and affective processes, aimed to neutralize the source of danger, through different behaviors such as fight or flight response, aggression, escape, etc. Involved in all these, monoamines and 5-HT act very rapidly, just within seconds after the onset of a stressor [3, 8–10]. Our hypothesis was that this rapid change in 5-HT levels could be detected and used for different forensic purposes.

In the presented study we investigated postmortem serotonin concentrations in cerebrospinal fluids (CSF) as a marker of its central activity in different manners of death, particularly in cases where the victims were aware of the stressful event.

Methods

Autopsy material

Our sample consisted of 81 autopsy cases, examined in the Institute of Forensic Medicine, Faculty of Medicine, University of Belgrade. The inclusive parameters for the analysis were: older than 18 years of age, with a postmortem interval (i.e. time from estimation of death until autopsy and sampling) within 72 h. Case profiles are presented in Table 1. Further inclusive parameters were: death at the scene without cardiopulmonal resuscitation and critical medical care before death; no documented history of either acute or chronic neuronal psychiatric or metabolic disorder, terminal diseases (as malignancy, e.g., and drug abuse or psychotropic therapy. Regarding surviving interval, determined as time between onsets of symptoms/injury to time of death, the distinction was made between very short and delayed (i.e. up to several minutes and up to several hours). Awareness of stressful life-threatening event was presented as dichotomy yes/no. In this regard, we divided the subjects to those who had been aware of impending lethal danger (either the one who was drowning or facing an attacker equipped with a gun or knife) and those who had no sense of it (the ones who died of heart failure during sleep or pedestrian hit from behind). The causes, manner of death and other data were determined through autopsy and toxicological findings while demographic data, circumstances and other data originated from police reports and heteroanamnesis.

Table 1

Demographic data and case profiles

Cause of death	Case number n (%)	Manner of death* N/A/S/H n (%)	Male/female n (%)	Survival time very short/delayed n (%)	Awareness of the stressful event yes/no n (%)
Violent death	50 (61.7)				
mechanical injury	17 (34)			13/4	17/0
blunt	7 (14)				
jump from a height	2	S (2)	2/0	2/0	2/0
traffic traumatism	4	A (4)	4/0	3/1	4/0
blows	1	H (1)	0/1	0/1	1/0
firearm	8 (16)	S (5)/H (3)	5/3	7/1	8/0
stabbing	2 (4)	h (2)	1/1	1/1	2/0
mechanical asphyxia	18 (36)				18/0
drowning	5	S (5)	3/2	5/0	5/0
hanging	13	S (13)	10/3	13/0	13/0
environmental injury	8 (16)				0/8
hypothermia	5	A (5)	1/4	0/5	0/5
electrocution	3	A (3)	3/0	3/0	0/3
CO intoxication	7 (14)	A (7)	4/3	5/2	3/4
Natural death					
acute cardiac death	31 (38.3)	N (31)	25/6	13/18	19/12
Total	81 (100)	31/19/25/6 (38.3/23.5/30.9/7.4)	58/23 (71.6/28.4)	52/29 (71.6/28.4)	57/24 (70.4/29.6)

***Manner of death: N – natural (38.3%); A – accidental (23.5%), S – suicidal (30.8%), H – homicidal (7.4%), out of total of violent deaths (61.7%).**
CO – carbon monoxide.

In order to evaluate relevance of CSF 5-HT levels, we related its values in central CSF with those from lumbar CSF as well as with its femoral serum levels. The specimens of CSF and serum were obtained during standard autopsy procedure. CSF samples were taken either after removing of calvaria and dura while brain was still *in situ* by needle puncture of ventral horn of a lateral ventricle through *corpus callosum* [11–13], while lumbar CSF samples were taken through ventral approach to lumbar cistern, puncturing intervertebral space L4/L5 after removal of abdominal content [7]. The samples of blood were taken through puncture of femoral vein, and then centrifuged to separate sera. Samples of the CSF and sera were immediately frozen and stored at -20°C until analyzed. To avoid influence of blood contamination or hemolysis only clear bright CSF and serum samples were analyzed, while those with cloudy or pinkish impurities were excluded.

Analytical procedure

In 100 μL of either CSF or serum sample, 20 μL of benzylamine, as internal standard, was added and briefly vortexed. Subsequently 30 μL of ethanol:pyridine solution (75:25, v/v) and 30 μL of reagent solution consisting of a mixture of ethylchloroformate, chloroform and n-hexane (in the ratio 20:70:10, v/v) was added for derivatization [14, 15]. The newly formed derivative was extracted with 500 μL of ethyl acetate, and then centrifuged (14,000 × g/15 min). Supernatant was transferred to a fresh vial and evaporated to dry under nitrogen flow. Dry extract was reconstituted with 60 μL of acetonitrile and placed into insert vial for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, conducted using Nexera UHPLC system coupled to liquid chromatography-mass spectrometry (LCMS)-8030 triple quadripole mass spectrometer (Shimadzu, Japan).

Statistical analysis

Group comparisons were obtained using parametric tests (Analysis of variance) and nonparametric (Mann-Whitney *U* test and Kruskall-Wallis test). The relationship between two variables was tested using Spearman's correlation. Receiver operating characteristic (ROC) analysis was used to estimate significant diagnostic test. Youden index was used to obtain an optimal cut-off point. All *p* values less than 0.05 were considered significant. The results are presented depending on data type as count (percent), medians (range) and mean (standard deviation - SD), in tables and box-plot (50% of the data are summarized in the box; the line in each box shows the median; lines outside of each box represent the 90% confidence interval). The obtained data were statistically analyzed using SPSS 20.0 (IBM corp.) and R for windows 3.3.0 (CRAN project) statistical software.

Results

The range of measurement for 5-HT concentrations was 0.08–143.6 ng/mL in all samples. The medians (range) and means ± SD of the 5-HT levels (ng/mL) for CSF and serum are presented in Table 2. There was no correlation between central CSF and peripheral serum levels, and central and lumbar CSF 5-HT levels (Table 3). Statistical significance was not found between the levels of the latter.

No gender-related differences of central CSF 5-HT levels were detected ($U = 553.000$; $p = 0.232$) (Table 3.). The subjects were 51.77 ± 14.26 years old (range 18-87 years). The differences in 5-HT levels among age groups were highly statistically significant ($\chi^2 = 13.354$; $p = 0.001$), with tendency to decrease with age. Even though levels of 5-HT increased with postmortem interval (Table 3), no statistically significant difference was detected ($\chi^2 = 1.572$; $p = 0.456$). The difference was not statistically significant between very short and delayed agony interval ($U = 708.000$, $p = 0.650$, Table 4). There was no statistically significant difference in 5-HT levels among subjects with and without awareness of lethal event ($U = 711.000$; $p = 0.775$, Table 4).

Table 2

Serotonin levels (ng/mL) in the cerebrospinal fluid (CSF) (central and lumbar) and serum

Body fluid	Median	Range (min–max)	Mean ± SD
Central CSF	2.75	0.08–6.13	2.86 ± 1.43
Lumbar CSF	3.01	1.44–7.36	3.26 ± 1.39
Serum	26.89	3.42–143.57	34.34 ± 29.72

SD – standard deviation.

Table 3

Correlations of serotonin levels (ng/mL) in the cerebrospinal fluid (CSF) and serum

Body fluid	Valid N	r	*p*
Central/lumbar CSF	17	- 0.015	0.955
Central CSF/ serum	46	- 0.095	0.530

Table 4

Serotonin levels in the cerebrospinal fluid (CSF) with regard to gender, age, postmortem interval, agony interval and awareness of the stressful event

Parameter	Serotonin levels in the central CSF (ng/mL)				*p* value
	valid N	median	range (min–max)	mean ± SD	
Gender					
male	58	2.84	0.08–6.13	2.97 ± 1.45	> 0.05
female	23	2.42	0.14–5.17	2.58 ± 1.36	
Age (years)					
< 40	16	3.66	1.85–6.03	3.81 ± 1.20	
41–65	52	2.75	0.08–6.13	2.81 ± 1.39	< 0.05
> 65	13	1.71	0.14–4.89	1.16 ± 1.71	
Postmortem interval (h)					
< 24	58	2.76	0.14–6.13	2.95 ± 1.40	> 0.05
25–48	15	2.75	0.08–3.62	2.33 ± 1.09	
49–72	8	3.42	0.61–5.23	3.22 ± 2.03	
Agony interval					
very short	52	2.72	0.14–6.13	2. 32 ± 1.5	> 0.05
delayed	29	2.84	0.08–5.59	2.73 ± 1.29	
Awareness of the stressful event					
yes	29	2.63	0.14–5.64	2.87 ± 1.51	> 0.05
no	51	2.88	0.08–6.13	2.9 ± 1.38	

SD – standard deviation.

Table 5

Serotonin levels in the cerebrospinal fluid (CSF) with regard to manner and cause of death

Parameters	Serotonin levels in the central CSF (ng/mL)				*p* value
	valid N	median	range (min–max)	mean ± SD	
Manner of death					
natural	31	2.50	0.08–4.72	2.32 ± 1.17	< 0.05
violent	50	3.13	0.14–6.13	3.2 ± 1.50	
accidental	20	3.56	0.14–5.64	3.38 ± 1.66	
suicidal	24	2.72	0.5–6.13	2.91 ± 1.3	> 0.05
homicidal	6	3.18	1.45–6.03	3.76 ± 1.73	
Cause of death					
cardiac	31	2.50	0.08–4.72	2.32 ± 1.13	
hanging	13	2.92	0.50–6.13	2.92 ± 1.52	
drawning	5	3.54	2.04–4.50	3.22 ± 0.99	
blunt injury	7	3.93	1.27–5.18	3.57 ± 1.20	
firearm injury	8	2.67	1.32–6.03	2.79 ± 1.55	> 0.05
stabbing	2	4.37	3.16–5.6	4.37 ± 1.72	
hypothermia	5	2.11	1.23–5.17	2.84 ± 1.56	
electrocution	3	3.51	2.63–5.30	3.82 ± 1.36	
intoxication	7	4.60	0.14–5.64	3.44 ± 2.16	

Concerning the manner of death, CSF 5-HT levels were significantly higher in violent deaths (U = 519.000, p = 0.013). The cut-off value for 5-HT levels in deaths from violent manner was 3.27 ng/mL, with a sensitivity of 46% and specificity of 87.1% (AUC = 0.665 (0.548–0.783); p = 0.013).

There was no statistically significant differences among subgroups of the violent manners (χ^2 = 1.765; p = 0.414), although a lower level of 5-HT was observed in the suicides (Table 5). Regarding the cause of death (Table 5, Figures 1 and 2), 5-HT levels were higher in blunt injury, stabbing and intoxications, while lower in cardiac deaths and hypothermia, however without statistically significant difference (F = 1.478, p = 0.180).

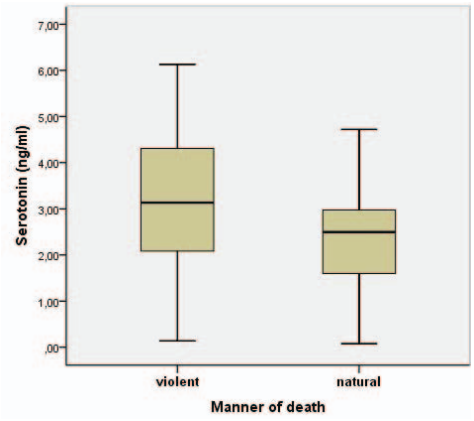

Fig. 1 – Box-plot representing differences in serotonin levels with regard to manner of death (natural/violent).

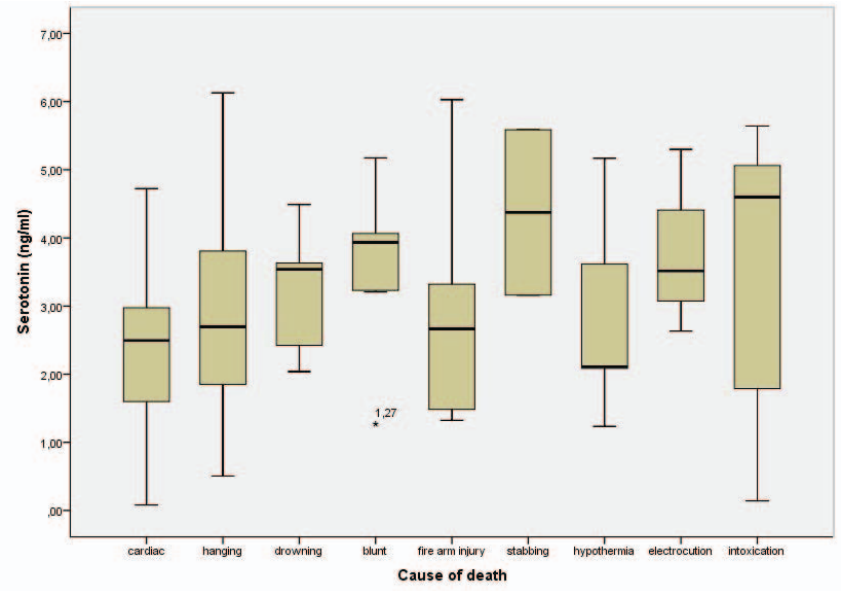

Fig. 2 – Box-plot representing differences in serotonin levels with regard to cause of death.

Discussion

Even if the role and importance of 5-HT were recognized decades ago, still its determination represents a great challenge in clinical and postmortem researches. Apart from interindividual variability in 5-HT activity, the postmortem 5-HT level depends upon many physiological body characteristics such as age, gender, weight, height of subjects, circadian rhythms of the 5-HT secretion, as well as postmortem conditions like postmortem interval, sampling and analytical methods, in detail summarized by Musshoff et al. [6]. For these reasons, there is still no consistency in referent 5-HT levels in literature, especially for its postmortem body fluids` values.

As a hydrophilic substance, 5-HT does not pass lipophilic blood-brain barrier readily, unlike its precursor tryptophan and metabolite 5-hydroxyindoleacetic acid. Hence, 5-HT in CSF mainly originates from the brain, while serum 5-HT is mainly derived from platelets [1, 16]. These mutually independent syntheses could explain the lack of correlation between central CSF and serum 5-HT levels in our results. Moreover, it could also mean that serum 5-HT levels do not reflect brain 5-HT neuroactivity.

The central and lumbar CSF compartments represent parts of a unique liquor system which circulates rostrocaudally through brain ventricles, central and spinal subarachnoidal space [17, 18]. For that reason, the lumbar approach to CSF system is widely used as a clinical diagnostic tool to reflect brain function [19]. Because of the rostrocaudal concentration gradient, in postmortem investigation of brain 5-HT, lumbar approach should not be the exclusive rule – two previous studies [6, 7] both revealed significantly higher values in central CSF. Our study showed no significant correlation between 5-HT central and lumbar levels, moreover, the median and mean levels in lumbar compartment were slightly higher. Excitation and firing activity in 5-HT neurons along the spinal cord, where it is involved in pain control [1, 20, 21], could contribute to such results. Further, our results could be due to differences among sampling sites in regard to postmortem degradation and/or because of diffusion from the surrounding tissues, certain in the process of postmortem decomposition.

What happens with 5-HT through the course of postmortem interval, apart from degradation/diffusion, is actually unclear – our study revealed the tendency of an increase in 5-HT levels, however without statistical significance. Previously, Quan et al. [5] observed the same, but with statistical significance.

The postmortem brain content of neurotransmitters can generally be determined through autopsy on two levels: in samples of brain tissue from exact brain areas and in samples of CSF which can be reached in its central compartments or by lumbar puncture [19]. It should be kept in mind, that CSF concentrations present only an average of neurotransmitters' levels from all brain regions in the moment of sampling and therefore cannot obtain information about functions of exact brain areas and neuronal groups. Even though the serotonergic system is involved in stress and worrying [22], and firing begins almost immediately**,** the previous might explain why 5-HT levels in CFS samples observed in the groups of very short and delayed interval between the onset of injury and moment of death, did not show any difference.

What we additionally tried to investigate herein is whether the magnitude of stress (caused by acute life threatening event) could somehow influence the 5-HT levels; for this purpose we divided subjects to those who were/were not aware of impending lethal danger. No differences were observed, moreover, mean values were very similar.

This study revealed significantly higher 5-HT levels in violent deaths than in natural ones. For this purpose, the cut-off value for distinguishing higher levels in violent manner was estimated at 3.27 ng/mL 5-HT. The area under the curve was small, but statistically significant. However, for the fur-

ther distinction among the groups of violent manner (i.e. accidental, homicidal and suicidal) our statistics did not reveal any significant differences, although with lower levels in suicides. Musshoff et al. [6] reviewed statistical significant decrease in 5-HT levels in suicides, while Kauert et al. [7] reviewed different.

Even though forensic approach to death clearly distinguishes mechanism of dying through different causes of death, our study on 5-HT CSF levels failed to prove these distinctions. Albeit the results apparently reveal lower 5-HT levels in cardiac death and hypothermia, higher in blunt injury and stabbing and the highest in carbon monoxide (CO) intoxication, there was a huge overlapping among these values. Unlike ours, previous studies showed remarkable and large differences among different causes of death [4–6].

Conclusion

The presented study determined central CSF as a body fluid of choice for postmortem measurements of serotonin neuronal activity. It also demonstrated that serotonin levels are markedly increased in violent deaths compared to natural ones, therefore serotonin could be a postmortem marker to distinguish natural and violent deaths, regardless of individual variability in concentrations.

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The significance of C-reactive protein for the prediction of net-adverse clinical outcome in patients with acute pulmonary embolism

Značaj C-reaktivnog proteina u predviđanju ukupnog nepovoljnog kliničkog ishoda kod bolesnika sa akutnom plućnom embolijom

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Abstract

Background/Aim. Acute pulmonary embolism (APE) may have different clinical manifestations. Also, its outcome can range from complete recovery to early death. Major bleeding (MB) as a due of the therapy also contributes to the overall adverse outcome. So far, it is unknown what the best predictors are for short-term mortality and MB among the several commonly used biomarkers. The aim of this study was to evaluate the significance of C-reactive protein (CRP) and other biomarkers for the prediction of adverse clinical outcomes. **Methods.** This clinical, observational, retrospective-prospective study included 219 consecutive adult patients treated for APE. **Results.** Among 219 patients, 22 (10%) died within the first month after diagnosis. Twenty seven patients (12.3%) had at least one episode of MB. Composite end-point [net-adverse clinical outcome (NACO)] was estimated in 47 (21.5%) of patients. The average values of all biomarkers were higher in the group of patient who died, and differences were statistically significant. Similar results were obtained for composite end-point. In terms of MB, none of biomarkers did not have significance, but CRP had a slight tendency toward significance. Results from univariate logistic regression model showed that troponin was statistically significant predictor of 30-day mortality. However, after adjusting for other variables, in multivariate logistic regression model troponin failed to be significant independent predictor of 30-day mortality. Unlike troponin, CRP and brain natriuretic peptide (BNP) were significant in all models – uni and multivariate (they were independent predictors of 30-day mortality). **Conclusion.** CRP has a good predictive value for 30-day mortality and NACO, and potential for MB in patients treated for APE.

Key words:
c-reactive protein; mortality; prognosis; pulmonary embolism; treatment outcome.

Apstrakt

Uvod/ Cilj. Akutna plućna embolija (APE) može imati različite kliničke manifestacije. Takođe, njen ishod može varirati od potpunog oporavka do rane smrti. Za sada nije poznato koji su najbolji prediktori kratkoročnog mortaliteta i velikih krvarenja među nekolicinom najčešće upotrebljavanih biomarkera. Cilj ovog istraživanja bio je da se utvrdi značaj C-reaktivnog proteina (CRP) i drugih biomarkera u predviđanju neželjenih kliničkih ishoda. **Metode.** Ova klinička, opservaciona, retrospektivno-prospektivna studija, obuhvatila je 219 uzastopnih odraslih bolesnika sa APE. **Rezultati.** Od 219 bolesnika 22 (10%) je umrlo unutar prvog meseca od postavljanja dijagnoze. Dvadeset sedam bolesnika (12.3%) imalo je najmanje jednu epizodu velikog krvarenja. Kompozitni cilj (ukupni neželjeni klinički ishod) utvrđen je kod 47 (21.5%) bolesnika. Srednje vrednosti svih biomarkera bile su veće u grupi umrlih bolesnika, a razlika je bila statistički značajna. Slični rezultati su utvrđeni za kompozitni cilj. U pogledu velikih krvarenja, nijedan biomarker nije pokazao značajnost, mada je CRP imao trend ka značajnosti. Rezultati univarijantne regresione analize pokazali su da je troponin značajan prediktor 30-dnevnog mortaliteta. Međutim, posle prilagođavanja sa drugim varijablama, multivarijantni logistički regresioni model nije potvrdio da je troponin značajan nezavisani prediktor 30-dnevnog mortaliteta. Za razliku od troponina, CRP i B-tip natriuretskog peptida (BNP) su

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značajni u svim modelima, univarijantnim i multivarijantnim (oni su nezavisni prediktori 30-dnevnog mortaliteta). **Zaključak.** Biomarker CRP ima dobru prediktivnu vrednost za 30-dnevni mortalitet i ukupan neželjeni klinički ishod, kao i potencijalnu prediktivnu vrednost za velika krvarenja kod bolesnika lečenih zbog APE.

Ključne reči:
c-reaktivni protein; mortaltiet; prognoza; pluća, embolija; lečenje, ishod.

Introduction

Acute pulmonary embolism (APE) may have different clinical manifestations, from asymptomatic to severe, life-threatening disease. Also, its outcome can range from complete recovery to early death. Major bleeding (MB) as a due of the therapy also contributes to the overall adverse outcome. In patients with massive pulmonary embolism (PE), the mortality rate is 18–65% (overall); about 20% in treated patients; 25–30% in patient with cardiogenic shock; 65% in patients with resuscitation. In submassive PE, the mortality rate is 5–25%; in PE with mobile thrombi in right-heart chambers – as high as 27% and in small PE up to 1% [1].

On the other hand, Chatterjee et al. [2] in meta-analysis reported that among patients with PE, including those who were hemodynamically stable with right ventricular dysfunction, thrombolytic therapy vs. anticoagulants was associated with lower rates of all-cause mortality (2.17% *vs* 3.89%) and increased risks of MB (9.24 *vs* 3.42%).

So far, it is unknown what the best predictors are for short-term mortality and MB among the several commonly used biomarkers in patients with APE. In the assessment of the early risk of death (in hospital and 30-day) from PE, in the prediction the severity and possible outcome in patients with PE, the most used parameters are D-dimer, cardiac troponin, natriuretic peptides [brain natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP)] [3–8]. Also, role of an inflammatory biomarker, C-reactive protein (CRP), as a predictor of prognosis in PE, was investigated [9]. Its role as a predictive biomarker of bleeding risk was investigated in other cardiovascular diseases. CRP is independent predictor of MB in elderly patients with myocardial infarction [10].

The aim of this study was to evaluate the significance of CRP and other biomarkers for the prediction of adverse clinical outcomes: 30-day mortality, MB and net-adverse clinical outcome (NACO – mortality plus MB) in patients treated for acute PE.

Methods

For the purpose of our research, we conducted a clinical, observational, retrospective-prospective study. Study was conducted at the Clinic for Emergency Internal Medicine, Military Medical Academy in Belgrade. We treated 243 patients with PE, but 24 patients were excluded because of underlying malignancy, so 219 patients were included in final analysis (116 men and 103 women). In all patients diagnosis was established with multidetector computed tomography with pulmonary angiography (MDCT PA).

Patients were treated according to local guidelines for pulmonary thromboembolism, which are in concordance to European guidelines [11, 12].

Inclusion criteria were as follows: age over 18 years, PE established with MDCT PA, first episode of PE, availability of a patient and his/her findings during the one month follow-up period.

Exclusion criteria were: pregnancy, active malignancy, active infection before PE (subsequent pneumonia due to PE was not exclusion criteria), known innate thrombotic conditions, existence of other conditions that may affect to hemostasis, previous treatment that can affect hemostasis (glycocorticoids, estrogen-progesterone drugs, testosterone, desmopressin), lack of data entering the study. Although the use of inclusion and exclusion criteria reduces the dimension of „real life", they are necessary in order to avoid confounding variables, for example, increasing mortality in case of active malignant diseases.

Variables that were determined: demographic parameters, comorbidities, biomarkers (CRP, BNP, troponin T), outcome – 30-day mortality, MB (according to the International Society of Thrombosis and Hemostasis definition)[13], NACO.

Statistical analysis

Data were analyzed by using IBM® SPSS® Statistics, release 23.0.0.2. Results are presented as count (percent), mean ± standard deviation or median (25th–75th percentile) depending on data type and distribution. Group comparisons were performed using Pearson χ^2 test, Fisher's exact test, *t*-test and Mann-Whitney *U* test. Logistic regression analysis was used to assess significant predictors of 30-day mortality. All *p* values less than 0.05 were considered significant.

Results

During the 10-year period we treated 219 patients with MDCT PA-confirmed PE. The baseline characteristics of enrolled patients are shown in Table 1.

The mean age of patients was 59 ± 17 years, ranging from 17 to 92 years. Distribution by gender was similar, as well as regarding spontaneous/provoked PE. The previous surgery (few months ago) had one fifth of patients. Also, one fifth of patients were active smokers. Arterial hypertension (AH) was the most common comorbidity. About half of the patients had high or intermediate-high risk. Simplified pulmonary embolism severity index (sPESI) score 1 or more was present in slight over half of the patients.

Adverse outcomes

Among 219 patients, 22 (10%) died within the first month after diagnosis. In 15 (68%) of them the cause of death was the APE, intracranial hemorrhage in 2 (9%) patients and other conditions in 5–23%. Twenty seven (12.3%) patient had at least one episode of MB. The most common bleeding site was gastrointestinal tract (11–45.9%); then surgery site (6–25%); urogenital tract (3–12.5%); intracranial (2–8.3%) and other (2–8.3%). Composite end-point (NACO) was estimated in 47 (21.5%) of patients.

Table 1

Baseline characteristics of patients

Parameters	Values
Age (years), mean ± SD	59 ± 17
Male, n (%)	116 (53)
BMI, mean ± SD	27.8 ± 4.8
Provoked APE, n (%)	101 (46.1)
Active smoking, n (%)	46 (21)
Surgery in last few months, n (%)	44 (20.1)
Comorbid conditions, n (%)	
COPD or emphysema	10 (4.6)
CHF	29 (13.2)
AH	93 (42.5)
CAD	18 (8.2)
DM	29 (13.2)
stroke (earlier)	14 (6.4)
Risk, n (%)	
low	71 (32.4)
intermediate-low	35 (16)
intermediate-high	79 (36.1)
high	34 (15.5)
sPESI score, n (%)	
0	89 (40.6)
≥ 1	130 (59.4)

BMI – body mass index; APE – acute pulmonary thromboembolism; COPD – chronic obstructive pulmonary disease; CHF – chronic heart failure; AH – arterial hypertension; CAD – coronary artery disease; DM – diabetes mellitus; sPESI – simplified Pulmonary Embolism Severity Index; SD – standard deviation.

As shown in Table 2, there was no statistically significant difference by gender, in terms 30-day mortality, MB and NACO. Mortality rate and NACO were higher in patients with provoked PTE than with spontaneous one, while the difference in the rate of MB was not statistically significant. Smoking habit had no impact on any adverse outcome in the study population, but it should be noted that the majority of the patients (79%) were nonsmokers. As regards comorbidities, univariate analysis was not indicated statistically significant difference for MB, while 30-day mortality and NACO were significantly higher in patients with concomitant diabetes, AH and stroke. In the other hand, chronic obstructive pulmonary disease and chronic heart failure did not showed influence on the 30-day mortality, while coronary artery disease showed a trend towards significance. Also, the 30-day mortality and NACO were significantly higher in the strata of the patients with high and intermediate-high estimated risk, than in low and intermediate-low strata, while regarding the MB there was not statistically significant difference.

As can be seen from Table 3, age was independent risk factor for the 30-day mortality and NACO. Body mass index did not show significance for any adverse outcome. Also, sPESI score was the independent predictor of mortality and NACO, but not of MB. The average values of all biomarkers were higher in the group of patient who died, and differences were statistically significant. Similar results were obtained for composite end-point. In terms of MB, none of biomarkers did not have significance.

Multivariate analysis was performed to rule out the influence of confounding variables (table 4). Results from univariate logistic regression model showed that troponin was statistically significant predictor of 30-day mortality. However, after adjusting for other variables, in multivariate logistic regression model troponin failed to be significant independent predictor of 30-day mortality. Unlike troponin, CRP and BNP were significant in both, uni and multivariate models. The R square of BNP was the largest in univariate, as in all other models (compared to CRP models).

Table 2

Correlation between the clinicodemographic factors and adverse outcomes

Parameter	Mort, n (%)	*p*-value	MB, n (%)	*p*-value	NACO, n (%)	*p*-value
Gender						
male	8 (6.9)	0.100	14 (13.0)	0.899	22 (19.0)	0.340
female	14 (13.6)		11 (12.4)		25 (24.3)	
APE						
spontaneous	6 (5.1)	0.008	13 (11.6)	0.600	18 (15.3)	0.016
provoked	16 (15.8)		12 (14.1)		29 (28.7)	
Active smoking						
no	21 (12.1)	0.088	17 (11.2)	0.369	39 (22.5)	0.531
yes	1 (2.3)		7 (16.3)		8 (18.2)	
COPD						
no	22 (10.5)	0.405	24 (12.8)	1.000	46 (22.0)	0.366
yes	0		1 (10.0)		1 (10.0)	
CHF						
no	17 (9.2)	0.541	19 (11.4)	0.230	38 (25)	0.223
yes	5 (14.3)		6 (20)		9 (31.0)	

Table 2 – *continued*

Parameter	Mort, n (%)	*p*-value	MB, n (%)	*p*-value	NACO, n (%)	*p*-value
CAD						
no	18 (9.0)	0.091	22 (12.0)	0.394	40 (19.9)	0.073
yes	4 (22.2)		3 (21.4)		7 (38.9)	
DM						
no	14 (7.4)	0.003	21 (11.9)	0.484	34 (17.9)	0.003
yes	8 (27.6)		4 (19.0)		13 (44.8)	
AH						
no	7 (5.6)	0.010	13 (10.9)	0.387	20 (15.9)	0.019
yes	15 (16.1)		12 (15.4)		27 (29.0)	
Stroke						
no	16 (7.8)	< 0.001	24 (12.7)	1.000	39 (19.0)	0.001
yes	6 (42.9)		1 (12.5)		8 (57.1)	
Risk						
low	1 (1.4)	< 0.001	8 (11.4)	0.157	8 (11.3)	< 0.001
intermediate-low	1 (2.9)		2 (5.9)		3 (8.6)	
intermediate-high	8 (10.1)		9 (12.7)		17 (21.5)	
high	12 (35.3)		6 (27.3)		19 (55.9)	

Mort – 30-day mortality; MB – major bleeding; NACO – net-adverse clinical outcome.
For other abbreviations see under Table 1.

Table 3

Clinical parameters and biomarkers related to the adverse outcomes (univariate analysis)

Parameter	30-day mortality		*p*-value	Major bleeding		*p*-value	Net-adverse clinical outcome		*p*-value
	no (n = 197)	yes (n = 22)		no (n = 192)	yes (n = 27)		no (n = 172)	yes (n = 47)	
Age (mean ± SD)	57.9 ± 17.2	68.6 ± 16.2	0.006	57.9 ± 17.4	57.8 ± 17.7	0.995	57.7 ± 17.4	63.55 ± 16.7	0.042
BMI (mean ± SD)	28.0 ± 4.9	26.1 ± 3.3	0.085	28.1 ± 4.7	27.7 ± 6.4	0.756	28.0 ± 4.7	27.2 ± 5.2	0.326
sPESI, Med ($^{25–75}$ perc.)	1 (0–2)	2.5 (1–3)	< 0.001	1 (0–2)	1 (0–2)	0.134	1 (0–2)	2 (1–3)	< 0.001
BNP, Med ($^{25–75}$perc.)	113.0 (42.8–285.5)	498.0 (267.6–988.0)	< 0.001	109.5 (39.7–252.0)	176.0 (74.0–360.0)	0.163	109.5 (39.3–254.6)	331.0 (120.5–506.0)	< 0.001
CRP, Med ($^{25–75}$perc.)	37.2 (18.1–93.0)	95.9 (57–155)	0.002	36.0 (17.95–80.5)	59.4 (21–133)	0.075	36.2 (17.6–80.5)	92.5 (34.4–145.8)	0.001
TnT, Med ($^{25–7p}$Perc.)	0.08 (0–0.46)	0.53 (0.07–1.10)	0.041	0.06 (0–0.45)	0.30 (0.03–0.60)	0.159	0.06 (0–0.44)	0.4 (0.06–0.90)	0.011

SD – standard deviation; Med – median; Perc – percentile; TnT – troponin T.
For other abbreviations see under Table 1.

Table 4

Results from multivariate logistic regression models – 30 day mortality

Parameter	BNP		CRP		Troponin T	
	p-value (R^2)	OR (95% CI)	*p*-value (R^2)	OR (95% CI)	*p*-value (R^2)	OR (95% CI)
No adj	< 0.001 (0.191)	1.002 (1.001–1.003)	0.009 (0.065)	1.007 (1.002–1.012)	0.052 (0.051)	2.192 (0.995–4.830)
Adj age	0.003 (0.240)	1.002 (1.001–1.003)	0.002 (0.204)	1.009 (1.003–1.016)	0.283 (0.231)	1.618 (0.672–3.893)
Adj gender	< 0.001 (0.199)	1.002 (1.001–1.003)	0.005 (0.091)	1.007 (1.002–1.013)	0.092 (0.111)	2.038 (0.891–4.662)
Adj age, DM, AH, stroke	0.005 (0.330)	1.002 (1.001–1.003)	0.003 (0.303)	1.010 (1.003–1.017)	0.279 (0.384)	1.678 (0.658–4.282)
Adj PESI 0/1+	0.004 (0.247)	1.002 (1.001–1.003)	0.020 (0.186)	1.007 (0.001–0.012)	0.297 (0.156)	1.548 (0.681–3.520)

BNP – B-type natriuretic peptide; CRP – C-reactive protein; OR – odds ratio; CI – confidence interval.
For other abbreviations see under Table 1.

CRP and BNP were independent predictors of 30-day mortality. Two models are not shown in Table 4, also showed significance of CRP as predictor of the 30-day mortality – CRP adjusted with risk (p = 0.011; R^2 = 0.239; OR 1.008; 95% CI- 1.002–1.014) and CRP adjusted with risk and age (p = 0.005; R^2 = 0.300 OR 1.010; 95% CI- 1.003–1.016).

Discussion

The aim of this investigation was to find the best biomarker for prediction of adverse outcome in patient treated for APE. While predictive value of troponin and BNP in APE is well known and validated, and they are implemented in guidelines [12], CRP was less investigated as a predictor of mortality in APE, but for the prediction of MB and NACO no relevant data exist in literature.

The role of systemic inflammation in cardiovascular diseases (CVD), including PE, is well known. On the one hand, various systemic inflammatory diseases (e.g. connective tissue diseases) could increase the risk for CVD [14]. Atherosclerosis, as pathological substrate for coronary artery disease, also presents an inflammatory disease. CRP, as surrogate marker of this process, plays role in the activation of the classical pathway of the complement system; increases low-density lipoprotein uptake into macrophages and enhances the ability of macrophages to form foam cells; inhibits endothelial nitric oxide synthase expression in endothelial cells; activates macrophages to secrete tissue factor, a powerful procoagulant; increases plasminogen activator inhibitor (PAI-1) expression and activity [15]. On the other hand, APE may induce local and systemic inflammatory response, by several mechanisms: ischemia, hypoxia, endothelial lesion, etc. These facts have been confirmed both in experimental models [16, 17], and in the study of Stewart et al. [18] where it is shown a partially reversible, systemic acute inflammatory response in APE, established by the significant increase of biomarkers (interleukin 6, CRP, myeloperoxidase and D-dimer) at diagnosis, followed by their decrease in more of 80% of patients over the next 3 months. As previously mentioned, inflammation may be cause or consequence of APE, but in addition, the reperfusion treatment could have the influence on the serum level of CRP [19]. Also, the role of inflammation and inflammatory markers in thrombus resolution and pulmonary vessels wall remodeling is described [20].

In our study, among biomarkers the best predictor of the 30-day mortality was BNP, than CRP, and troponin T. Good predictive value of CRP for the 30-day mortality was shown in both, univariate and multivariate analyses, where CRP was adjusted with some demographic (age, gender) or clinical (comorbidity) factors which were significant in the univariate analysis (CRP and BNP were independent predictors of the 30-day mortality). None of biomarkers did not show predictive value for MB, but CRP had a slight tendency toward significance ($p = 0.075$), so further research on a larger sample is needed to confirm its value. Regarding the composite end-point (NACO), similar results were obtained as for the 30-day mortality.

In respect of clinicodemographic factors, in our study independent predictors of the 30-day mortality and NACO were age, provoked APE, diabetes, AH and stroke. Age is implemented as prognostic factor in both the PESI and sPESI score. Role of comorbidity is also well known, and our results are in concordance with previous investigations. Polo Friz et al. [21] were estimated that in elderly patients with a hemodynamically stable PE, and confirmed the Charlson Comorbidity Index score as an independent predictor of mortality. In our investigation the 30-day mortality was three times higher in patients with provoked pulmonary tromboembolism than with spontaneous one, while NACO was twice higher, and the difference for both variables was highly significant. Gjonbrataj et al. [22] conducted study in Korea and established that the 30-day mortality was 2-fold higher in patients with provoked PE than in those with unprovoked PE, although that difference was not statistically significant.

Also, higher sPESI score, high and intermediate-high estimated risk are confirmed as predictors of the 30-day mortality and NACO, which is consistent with the literature data [23–25].

Conclusion

CRP has a good predictive value for the 30- day mortality and NACO, and potential for prediction of MB in patients treated for APE.

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Correlations between symptoms, nasal endoscopy and computed tomography findings in patients with chronic rhinosinusitis without nasal polyps

Korelacije između simptoma, nalaza endoskopije nosa i kompjuterizovane tomografije kod bolesnika sa hroničnim rinosinuzitisom bez nosne polipoze

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Abstract

Background/Aim. Chronic rhinosinusitis (CRS) is one of the most common chronic conditions that is diagnosed on the basis of the condition symptoms, nasal endoscopy and computed tomograhpy (CT) of the nose and paranasal sinuses. There are two forms of CRS: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). The aim of this paper was to determine if there is a correlation between the symptoms, nasal endoscopy and CT in patients with CRSsNP. **Methods.** The study included 110 patients with CRSsNP. The intensity of the symptoms assessed on the visual analogue scale (VAS) and the condition of nasal mucosa and the presence of nasal secretion was estimated by endoscopic examination of the nose while CT was used to determine the Lund-Mackay (LM) score values. Pearson's coefficient of correlation was used for statistic data processing. **Results.** The severity of the disease as a whole (r = 0.509) and nasal discharge (r = 0.562) moderately correlated with CT. Nasal congestion (r = 0.354) and the reduction of loss of smell (r = 0.324) mildly correlated with CT, while facial pain/pressure (r = 0.218) had a very weak correlation with CT. The severity of the disease as a whole (r = 0.717) and nasal discharge (r = 0.821) strongly correlated with nasal endoscopy. Nasal congestion (r = 0.525) had a moderate correlation with nasal endoscopy while facial pain/pressure (r = 0.345) and the reduction of the loss of smell (r= 0.394) had a mild correlation with nasal endoscopy. A moderate correlation was found between nasal endoscopy and CT (r = 0.630). **Conclusion.** The severity of the disease as a whole and nasal discharge have more significant correlations both with nasal endoscopy and CT in relation to the correlations between other symptoms and nasal endoscopy, as well as CT. More significant correlations between the symptoms and nasal endoscopy in relation to the correlations between the symptoms and CT and the existence of a moderate correlation between nasal endoscopy and CT, enable a lesser use of CT diagnostics and only in precisely defined situatiations.

Key words:
chronic disease; endoscopy; rhinitis; severity of illness disease; signs and symptoms; sinusitis; tomography, x-ray computed.

Apstrakt

Uvod/ Cilj. Hronični rinosinuzitis (HRS) je jedno od najčešćih hroničnih oboljenja koje se dijagnostikuje na osnovu simptoma bolesti, endoskopije nosa i kompjuterizovane tomografije (KT) nosa i paranazalnih sinusa. Postoje dve forme rinosinuzitisa: HRS sa polipozom nosa (HRSsPN) i HRS bez polipoze nosa (HRSbPN). Cilj rada je bio da se utvrdi da li postoje korelacije između simptoma, nalaza endoskopije nosa i KT kod bolesnika sa HRSbPN. **Metode.** Istraživanjem je obuhvaćeno 110 bolesnika sa HRSbPN. Intenzitet tegoba je bio procenjen na vizuelnoj analognoj skali (VAS); endoskopskim pregledom nosa procenjeno je stanje nosne sluznice i prisustvo sekreta u nosu, a korišćenjem KT nosa i paranazalnih sinusa utvrđene su vrednosti Lund-Mackay skora. Za statističku obradu podataka korišćen je Pirsonov koeficijent korelacije. **Rezultati.** Težina bolesti u celosti (r = 0,509) i sekrecija iz nosa (r = 0,562) umereno su korelirale sa vrednostima KT skora. Zapušenost nosa (r = 0,354) i osećaj oslabljenog ili izgubljenog mirisa (r = 0,324) blago su korelirali sa vrednostima KT skora, dok je korelacija između osećaja bola/pritiska u licu (r = 0,218) i KT skora

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bila vrlo slaba. Težina bolesti u celosti (r = 0,717) i sekrecija iz nosa (r = 0,821) snažno su korelirale sa nalazima endoskopije nosa. Zapušenost nosa (r = 0,525) je imala umerenu korelaciju sa nalazom endoskope nosa, dok su osećaj bola/pritiska u licu (r = 0,345) i osećaj oslabljenog ili izgubljenog mirisa (r = 0,394) blago korelirali sa nalazom endoskopijom nosa. Korelacija između nalaza endoskopije nosa i KT bila je umerena (r = 0,630). **Zaključak.** Težina bolesti u celosti i sekrecija iz nosa, u odnosu na druge tegobe, značajnije koreliraju, kako sa nalazom endoskopije nosa tako i sa KT skorom. Značajnije korelacije između simptoma i nalaza endoskopije nosa u odnosu na korelacije između simptoma i KT skora, uz postojanje umerene korelacije između nalaza endoskopije nosa i KT skora, omogućavaju da se KT nosa i paranazalnih sinusa koristi samo u jasno definisanim situacijama.

Ključne reči:
hronična bolest; endoskopija; rinitis; bolest, indeks težine; znaci i simptomi; sinuzitis; tomografija, kompjuterizovana, rendgenska.

Introduction

Chronic rhinosinusitis (CRS) is a very common chronic condition taking the second place on the list of all chronic conditions in the USA [1]. It impairs the quality of life to a great extent and it has been proven that this condition has a bigger impact on patients' social interaction in comparison to heart disease, angina or back pain [2]. CRS has a great impact on patients' health as well as the health and economic system of one country. The costs for curing this condition, both directly and indirectly in the USA, place the condition among the first ten conditions with the biggest annual costs [3]. Almost 85% of CRS patients is aged between 18 and 65, which is the prime time of the working life [4].

The diagnosis of CRS at the tertiary health level is made on the basis of the symptoms, and it is confirmed by nasal endoscopy and/or computed tomograhpy (CT) of the nose and the paranasal sinuses. There are two forms of CRS: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). Albeit these two forms have a lot in common and mutual interfering, they differ to a certain extent in regard to inflammatory profile, clinical manifestations and therapeutic effects [5]. A significant number of patients with CRS is cured on the primary health level, where the diagnosis is made on the basis of the symptoms. At this level physicians have no experience nor adequate equipment for nasal endoscopy [6], and no possibilities for costly CT diagnostics [7]. This can lead to wrong diagnoses, which can further lead to an inadequate treatment with all the consequences that can arise from it. However, the symptoms are the main diagnostic procedure of CRS, because they are less time-consuming in everyday work enabling a valuable understanding of the condition as well as the assessment of the effect of the administered therapy [8].

Numerous authors have researched if there is a correlation between subjective symptoms and objective disease parameters in CRS patients with the aim to obtain a much simpler, faster, cheaper, and, which is the most important fact, more reliable way to make a right diagnosis and decide on a right and timely treatment, avoiding all unwanted effects of consequences of the mistakes in diagnostics and treatment. The results of these researches are controversial [9].

The aim of this paper was to determine if there is a correlation between a subjective symptom severity assessment, nasal endoscopy and CT findings in patients with CRSsNP.

Methods

This study involved 110 patients (47 men and 63 women), aged between 18 to 81. They were all dignosed with CRSsNP on the basis of the clinical symptoms according to the European Position Paper on Rhinosinusitus and Nasal Polyps 2012 (EPOS 2012) criteria. The symptoms lasted more than 12 weeks in all patients. They all had previous primary and/or secondary health level medical treatments that lasted at least six weeks. Some of them were treated at a tertiary health level by otorhinolaryngologists who are not experts on rhinology. Since the results of the treatments were not satisfying, the patients were sent to specialists on rhinology to be reviewed for a surgery. The patients with allergic rhinitis, CRSwNP, benign or malignant tumors in the nose, acute infections of the upper respiratory tract and the patients who had had nasal or paranasal surgical procedures were excluded from the study. Also, the patients with chronic headaches, cistic fibrosis, immunocompromised conditions, pregnant women, breastfeeding women and subjects younger than 18 were also excluded. The research was done in accordance with the principles of the Helsinki declaration and it was approved by a local ethical committee. All the patients were thoroughly informed on the procedures of the study and gave a written consent to participate in it after fully understanding it. They were free to leave at any point of the study without any influence on their further treatment and the way they were treated.

Each patients' symptoms were recorded (nasal congestion, nasal discharge, facial pain /pressure, reduction or loss of smell). Intensity of every symptom was assessed on the visual analogue scale (VAS) from 0–10, with 0 indicating that there was no symptoms, and 10 the maximum intensity of the symptoms. Also, by using the same scale, the patients assessed the severity of the disease as a whole 0–10 cm. During the same examination every patient underwent nasal endoscopy with the endoscope of 30 degrees 4 mm. The state of nasal mucosa and the quantity of secretion were observed. The state of nasal mucosa in relation to the existence of edema for both parts of the nose was assessed as follows: 0 – absence of edema, 1 – mild and 2 – severe edema. The maximum value for this parameter is 4. The amount of secretion for each side of the nose was assessed as follows: 0 – no secretion, 1 – clear, thin secretion, 2 – thick, purulent nasal secretion. The maximum value of this parameter is 4 as well.

The states of nasal mucosa on both sides and the secretion on both sides were added up and the obtained result was considered as the endoscopic finding of the nose with the maximum value of 8. CT scanning of the nose and paranasal sinuses were done after collecting the data on the symptoms and nasal endoscopy in the period between 2 and 4 days on multiple detector (MD) CT Siemens devices, models Sensatin 64 and Emotion 16, by a series of direct axial cross-sections with reconstructing intervals of 1 mm and multiplanar reconstruction (MPR) coronal cross-sections with reconstructive intervals of 2 mm. By using Lund-Mackay (LM) scale for every paranasal sinus on both sides (maxiallary, frontal, anterior ethmoids, posterior ethmoids and sphenoid), the state of sinusal opacification was assessed from 0 – no opacification, 1 – partial opacification, 2 – total opacification (for both ostio-meatal complexes 0 – non occluded, 2 – occluded). The maximum value of CT LM score is 24.

After collecting all the data, the correlations between every individual symptom severity and CT findings were examined as well as the correlations between the severity of the disease as a whole and CT finding of the nose and paranasal sinuses. Afterwards, the correlations between the severity of every individual symptom and endoscopic nasal findings were examined as well as between the assessment of the severity of the disease as a whole and endoscopic nasal findings. At the end, the correlation between nasal endoscopy and CT finding of the nose and the paranasal sinuses was examined.

Numerical data are presented as measures of central tendency (mean, median) and the measures of variability (standard deviation, minimum, maximum). The connection testing between two properties was done by using Pearson's coefficient of correlation (r). Statistical analysis was performed using IBM SPSS Statistics 21.

Results

Out of total of 110 examined patients with CRSsNP in this paper there were 63 (57.27%) women and 47 (42.73%) men with the ratio of 1.34 : 1. The average age of the examined patients was 42.09 years, ranging between 18 and 81 years of age.

The prevalence of certain symptoms, the mean values of symptoms severity and the disease as a whole, the values of endoscopic score and CT LM score as well as their standard deviations, minimums, maximums and ranges are shown in Table 1. The assessment of the disease severity as a whole with the average value of 4.91, nasal discharge 5.15, nasal congestion 4.29 and the sensation of facial pain/pressure 3.08 belonged to moderate symptoms, while reduction or loss of smell with the mean value of 2.23 belonged to mild symtoms.

The severity of the disease as a whole (r = 0.509) and nasal discharge (r = 0.562) moderately correlated with CT values. Nasal congestion (r = 0.354) and the reduction of loss of smell (r = 0.324) mildly correlated with CT values, while facial pain/pressure (r = 0.218) had a very weak correlation with CT values (Table 2).

The severity of the disease as a whole (r = 0.717) and nasal discharge (r = 0.821) strongly correlated with nasal endoscopy. Nasal congestion (r = 0.525) had a moderate correlation with nasal endoscopy finding while facial pain/pressure (r = 0.345) and the reduction of the loss of smell (r = 0.394) had a mild correlation with this finding (Table 3).

Table 1
The values of symptom intensity and the disease as a whole, the values of nasal endoscopic and computed tomography Lund-Mackay (CTLM) scores in patients with chronic rhinosinusitis (n = 110)

Parameters	Mean ± SD	Median	Minimum–Maximum
Disease as a whole	4.91 ± 2.118	5	1–10
Nasal congestion	4.29 ± 2.006	4	0–10
Nasal discharge	5.15 ± 2.605	5	0–10
Facial pain/pressure	3.08 ± 2.638	4	0–10
Reduction or loss of smell	2.23 ± 2.459	2	0–10
State of nasal mucosa	2.09 ± 1.088	2	0–4
Nasal secretion	1.11 ± 1.061	1	0–4
Nasal endoscopic score*	3.2 ± 1.999	3	0–8
CT LM score	9.37 ± 5.312	8	1–22

Note: The values of the state of nasal mucosa and the values of the nasal secretion were added up and the obtained result was considered as nasal endoscopic score.
SD – standard deviation.

Table 2
Correlations between symptoms and computed tomography finding in patients with chronic rhinosinusitis (n = 110)

Parameters	Disease as a whole	Nasal congestion	Nasal discharge	Facial pain/pressure	Reduction or loss of smell
r	0.509	0.354	0.562	0.218	0.324
p	0.000	0.000	0.000	0.022	0.001

***r* – Pearson's correlation coefficient; *p* – statistical significance.**

Table 3
Correlations between symptoms and nasal endoscopy finding in patients with chronic rhinosinusitis (n = 110)

Parameters	Disease as a whole	Nasal congestion	Nasal discharge	Facial pain/pressure	Reduction or loss of smell
r	0.717	0.525	0.821	0.345	0.394
p	0.000	0.000	0.000	0.000	0.000

***r* – Pearson's correlation coefficient; *p* – statistical significance.**

A moderate correlation was found between nasal endoscopy and CT score ($r = 0.630$), (Figure 1).

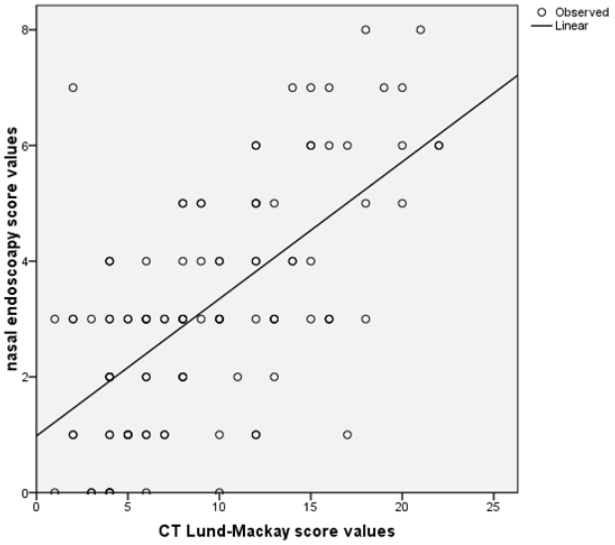

Fig. 1 – Correlation between findings of nasal endoscopy and computed tomography (CT) in patients with chronic rhinosinusitis (n = 110).

Discussion

CRS is a greatly widespread condition with the prevalence of 15.5% in the United States [1]. The European prevalence is 10.9%, depending on every country individually ranging from 6.9% to 27.1% [10]. Besides the EPOS guidelines for making a diagnosis of CRS in 2012 in everyday practice, certain unclarities and misdiagnoses arise, which leads to wrong treatments. There are numerous reasons for that; considering a great number of such patients and diverse systems of health care organizations of certain countries, these patients are diagnosed and treated at different levels of health care at general practitioners, pediatricians, specialists in internal medicine, allergologists, otorhinolaryngologists and others. At every level of health care, there is different knowledge and different experience in diagnosing and treating of CRS, unequal availability of diagnostic procedures such as nasal endoscopy and CT diagnostics of the nose and paranasal sinuses. Also, patients are often unable to distinguish clearly the symptoms of this condition, they often mix the sensations of nasal congestion and facial pain/pressure [11]. Patients often mistake headaches that are usually of neurogenous nature for CRS and they describe them as facial pain/pressure [5]. The symptoms that characterize CRS can occur in patients with infections of upper respiratory tract, allergic rhinitis and other rhinological ailments [11]. All this, if not scrutinized, can lead to mistakes in dignostics and treatment of this condition, and mistakes can occur when examining the correlations of the symptom severity and nasal endoscopy and CT scanning of the nose and paranasal sinuses, which all can lead to a wrong estimation of their values when diagnosing CRS.

The average age of the examined patients in our paper was 42.09 ranging from 18 to 81 years of age, which is very similar to the researches of other authors [12–15]. The examinees in the research of Ryan et al. [11] were slightly older with the average age of 51, while the average age of examinees in the papers of Pokharel at al. [7] Nair [16], and Gairolae at al. [17] was about 32 years of age. There were more female than male patients in this study with the ratio of 1.34 : 1. The majority of female patients was also found by other authors examining the correlations of subjective and objective findings in patients with CRS [7, 14–16]. On the other hand, Deepthi at al. [12], Birch at al. [13] and Gairola et al. [17] had significantly more male patients in their studies. These data on the age and gender can be of significance, because they can have an impact on the subjective sensation of the disease severity [5]. Baumann et al. [18] have found that female CRS patients have the values of the 20-Item Sino-Nasal Outcome Test (SNOT-20) significantly higher than male patients, while the severity of the condition determined by imaging diagnostics was the same in both genders. Also, subjective assessments of the disease severity can be influenced by cultural and ethnic characteristics of the patients as well their socioeconomic status along with their comorbities and other factors [5].

Previous studies on correlations between subjective assessment of the disease severity and CT score in CRS patients differ greatly. Our results showed that there are moderate correlations between a subjective assessment of the disease severity as a whole and nasal discharge, which is the most intense symptom in patients in our study, and CT finding. The correlations between nasal congestion and reduction of loss of smell and CT finding were mild, while the correlation between facial pain/pressure and CT finding was very weak. Kenny et al. [14] have found the results mildly similar to ours with a positive correlation between all the symptoms (of diverse degrees of significance), as well as the total symptom score and CT findings, except between the sensation of facial pressure and headache and CT finding, where there is no significant correlation. Numerous studies have found significant correlations between symptom severity assessment and CT LM score [7, 12, 16, 19]. On the other hand, there are numerous studies which found a partial or no correlation between

these two parameters. Basu et al. [20] as well as Holbrook et al. [21] have found that there is no correlation between a subjective assessment of the disease severity and CT LM score in CRS patients, while Holbrook et al. [21] state that there is no correlation between the opacificationing region on CT scans and the region where the patients feel actual facial pain. Wabnitz et al. [22] have found that there is no correlation between individual symptom severity characterizing CRS and CT findings, but when the whole symptom score and CT LM score are observed, there is a weak but statistically significant correlation between them. Rosbe and Jones [23] have shown that, only when dealing with nasal congestion as the major symptom, CT findings confirm CRS, while the patients complaining about facial pain/pressure most probably do not have CRS. The differences in the results regarding these two parameters, besides the mentioned demographic characteristics of the examinees, have other reasons. The degree of symptoms plays an important role (mild, moderate, heavy) [5] – the heavier the symptoms, the bigger the probability of the correlation between them and CT findings [7, 21]. Also, the time that passes between a subjective assessment of the symptom severity and doing CT scanning is very important (whether it was simultaneously, or the gap is expressed in days, sometimes weeks). Considering the course of the disease, the longer the period between these diagnostic procedures, the weaker the validity of the correlation. Also, the results of these studies depend on the kind, notably the sensitivity of the test implemented for the subjective assesment of the symptom severity and also if the study excluded the patients with nasal polyposis or not.

The results of this study showed strong correlations between the severity of the disease as a whole and nasal discharge, as the most intense symptoms in the patients of this study, and nasal endoscopy finding. Nasal congestion had a moderate correlation with nasal endoscopy finding while reduction or loss of smell and facial pain/pressure have mildly correlated with this finding. More significant correlations that we found between symptoms and nasal endoscopy finding in relation to the correlations between symptoms and CT score, could be explained by a better observing nasal mucosal edema by nasal endoscopy than by using CT [24]. In this regard, nasal endoscopy enables also a better assessment of nasal secretion amount than CT. Also, in about 30% of asymptomatic adult examinees, there are incidental mucosal changes on CT [25]. Numerous studies have found a significant correlation between the symptoms and nasal endoscopy findings [7, 12, 19]. On the other hand, Liu et al. [26] have found that only weakened or lost olfactory function is in correlation with endoscopic findings, while between other symptoms, as well as the total subjective score of the disease and endoscopic findings, there is no significant correlation in CRS patients. Birch et al. [13], as well as Gairola et al. [17], did not find any kind of correlation between a subjective condition severity and endoscopic finding in these patients. Gairola et al. [17] state that the most probable reason for that is CRS patients overestimate the severity of their symptoms. Other reasons of these contradictory findings that arise when comparing these two parameters could be explained in a similar way as the contradictions of the results comparing a subjective assessment of the condition severity and CT finding, as mentioned before.

Although doing a CT scanning of the nose and the paranasal sinuses is considered a “golden standard” in diagnosing CRS, this method is very expensive and not always feasible, and patients undergo a significant amount of radiation. On the other hand, nasal endoscopy is relatively simple and not so expensive, fast to perform, moderately sensitive and very specific in predicting the results of CT findings [23]. The results of this research have shown that there is a moderate correlation between nasal endoscopy findings and CT LM score in patients with CRSsNP. Most authors state that there is a significant correlation between nasal endoscopy and CT findings [7, 12, 27, 28]. This is the very reason why Bhattacharyya and Kepnes [3] and the Lohiya et al. [29] recommend using medicamentous therapy for CRS in patients with positive symptoms and positive endoscopic results, and doing diagnostic CT only in the patients whose symptoms persist after maximum medicamentous therapy. Also, diagnostic CT should be done in patients with positive symptoms and negative endoscopic findings. Ryan et al. [11] have found a very high degree of correlation between endoscopic and CT findings in the patients after endoscopic sinusal surgical procedure and explained that these patients have a significantly better visibility of sinusal cavities in relation to nonsurgical patients. On the basis of these findings, they recommended endoscopy as the main diagnostic procedure in the patients whose symptoms persist after endoscopic surgical procedure. On the contrary of most studies, Stankievicz and Chow [30] have found a very poor correlation between nasal endoscopy and CT findings with nasal endoscopy having sensitivity of 46%, specificity of 86%, positive predictive value 74% and negative predictive value of 64%.

Conclusion

The severity of the disease as a whole and nasal discharge have more significant correlations both with nasal endoscopy and CT score in relation to the correlations between other symptoms and nasal endoscopy and CT score. Facial pain/pressure in relation to other symptoms correlate in a very weak way with nasal endoscopy and especially with CT score. More significant correlations between the symptoms and nasal endoscopy in relation to the correlations between the symptoms and CT score and the existence of a moderate correlation between nasal endoscopy and CT score, enable CT diagnostics to be used only in patients who do not respond to medical therapy, and also in the patients in whom there is no correlation between the symptom severity and nasal endoscopy, as well as in patients who are on the waiting list for surgical treatments.

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Clinical and electrophysiological features of peripheral neuropathy in older patients with lung carcinoma

Kliničke i elektrofiziološke karakteristike periferne neuropatije kod starijih bolesnika sa karcinomom pluća

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Abstract

Background/Aim. Peripheral nervous system affection in people with lung cancer is commonly associated with paraneoplastic neuropathy. However, clinical studies evaluating the frequency, clinical, and electrophysiological characteristics of peripheral neuropathies which are not related to onconeuronal antibodies, in this, on average, older population of patients, are very rare. The aim of this study was to define the frequency, as well as clinical and electrophysiological characteristics of idiopathic neuropathies in patients suffering from lung cancer in early stages of the diseases. **Methods**. Clinical and electrophysiological data of 105 elderly subjects (age 63.4 ± 7.8 years) suffering from lung carcinoma who underwent extensive neurological and electrophysiological evaluation (nerve conduction studies) between 2013–2018 were estimated. Exclusion criteria were "classical" paraneoplastic neurological syndromes with onconeuronal antibodies present, as well as patients with typical known causes of peripheral neuropathy (e.g. diabetes, alcoholism, chronic renal insufficiency, vitamin deficiencies, etc.). **Results**. There were 19.1% patients with clinically manifest neuropathies, with additional 37.1% patients with only electrophysiological abnormalities. The most frequent pathophysiological pattern was axonal pathology (71.2%) with predominantly distal and symmetrical distribution (86.4%). **Conclusion**. Patients with lung cancer in the early stages of the disease show a high incidence of clinically minor damage of the nerves, according to the pattern of chronic sensomotor distal neuropathy, with predominance of axonal damage. These findings underline the importance of a detailed clinical and electrophysiological evaluation in this category of patients who are without the typical etiological factors for peripheral neuropathies since, during cancer therapy, patients undergo a series of treatments with additional risk for the development/aggravation of neuropathy.

Key words:
lung neoplasms; polyneuropathies; comorbidity; aged; electrophysiology.

Apstrakt

Uvod/Cilj. Zahvatanje perifernog nervnog sistema kod osoba obolelih od karcinoma pluća uobičajeno se povezuje sa paraneoplastičkim neuropatijama. Studije koje procenjuju učestalost, kliničke i elektrofiziološke karakteristike perifernih neuropatija koje nisu povezane sa onkoneuronalnim antitelima, kod ove populacije bolesnika, u proseku starije životne dobi, veoma su retke. Cilj ove studije bio je da se definišu učestalost, kao i kliničke i elektrofiziološke karakteristike idiopatskih neuropatija kod bolesnika sa karcinomom pluća u ranim stadijumima bolesti. **Methods**. Prikazani su klinički i elektrofiziološki podaci o 105 starijih bolesnika (63,4 ± 7,8 godina) obolelih od karcinoma pluća koji su bili podvrgnuti detaljnoj neurološkoj evaluaciji i ispitivanju provodljivosti nerava između 2013. i 2018. godine. Bolesnici sa "klasičnim" paraneoplastičnim neurološkim sindromima uz prisustvo onkoneuralnih antitela, kao i oni koji boluju od poznatih uzroka periferne neuropatije (npr. dijabetes, alkoholizam, hronična bubrežna insuficijencija, deficijencija vitamina D) isključeni su iz studije. **Rezultati.** U ovom istraživanju 19,1% bolesnika imalo je klinički manifestnu neuropatiju, a dodatnih 37,1% imalo je samo abnormalnosti uočene tokom ispitivanja nervne provodljivosti. Najčešći patofiziološki supstrat bila je aksonalna patologija (71,2%) sa predominantno distalnom i simetričnom distribucijom (86,4%). **Zaključak.** Bolesnici oboleli od karcinoma pluća u ranoj fazi bolesti pokazuju visoku učesta-

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lost klinički nemih oštećenja nerava, a prema obrascu hronične senzori-motorne distalne neuropatije lakog stepena ispoljavanja, uz predominaciju aksonalnih oštećenja. Ovi rezultati ukazuju na značaj detaljne kliničke i neurofiziološke evaluacije ove kategorije bolesnika bez tipičnih etioloških faktora za periferne neuropatije, budući da se tokom lečenja bolesnici podrgavaju nizu tretmana sa dodatnim rizikom od razvoj/pogoršanja neuropatija

Ključne reči:
pluća, neoplazme; plineuropatije; komorbiditet; stare osobe; elektrofiziologija.

Introduction

Malignant lung tumors represent a leading cause of mortality in the world [1]. Therefore, early detection techniques pose an essential field of interest, especially having in mind that this tumor is often diagnosed after it has metastasized [2]. Besides the "classical" metastasis syndrome, there are other clinical presentations which can represent an effect of the tumor, and these effects are especially prevalent in the structures of the nervous system. Having in mind the general population, the paraneoplastic neurological syndromes (PNS) represent equally rare symptoms, mediated by onconeural antibodies and depends highly on the type of cancer [3].

Recent research has shown that the manifestations of the PNS on the peripheral nervous system is most common in small-cell lung cancer [4, 5], although rare even in this type of cancer [6]. Contrary to patients in the early stages of the disease, if the frequency is examined in patients in late stages, the damage of the peripheral nervous system is present in as much as 15% of patients, where this is pathophysiologically explained as metabolic and nutritive deficiencies, and/or the effects of chemotherapy as part of the primary disease treatment [7].

In cases of early PNS manifestations, or even as a first simptom of a lung tumor (as well as some other solid organ tumors), these effects are explained by the presence of typical onconeural antibodies (anti-Hu, Yo,Ri, CV_2/$CRMP_5$, Ma_2, andantiamphiphysin) in the serum of lung cancer patients. Therefore, following the recommendations of the International Panel of Neurologists, it is assumed that the two levels of evidence confirming the diagnosis of PNS as "definitive" or "possible", which is dependant on the presence of onconeural antibodies [8], mostly is based on the clinical manifestations – classical versus non-classical forms of neuropathy [3].

Nevertheless, considering that lung cancer is typically a disease of the elderly, around 70 years of age on average [9], a higher frequency of neuropathies in this population, independently of PNS, is already present, compared to a younger demographic. This frequency has been seen to arrive to up to 7% in some studies of the elderly population [10, 11]. Among the detected neuropathies, there is a notable fraction, reported going to up to 49% in elderly patients, of characteristical cryptogenic, or chronic idiopathic axonal neuropathies [12].

Having in mind the fact that older patients suffer from lung cancer more often, and equally, but not necessarily causally connected, a significant fraction of elder patients present with neuropathic symptoms, a clinical interest in defining the frequency, as well as clinical and electrophysiological characteristics of idiopathic neuropathies in patients suffering from lung cancer in early stages of the diseases, is evident.

Methods

Patients

Between December 2013 and January 2018, 105 patients with pathologically confirmed lung carcinoma were referred to our clinic regarding the screening neurological and electrophysiological evaluation aimed to diagnose peripheral neuropathy. For this study, we selected only those patients who had no other known causes of peripheral neuropathy, including classical paraneuroplastic peripheral neuropathy, but diabetes mellitus, long-term alcohol consumption, renal failure, vitamin deficiencies, thyroid gland dysfunction, paraproteins, and cachexia, as well.

The study protocol was approved by the Ethical Committee of Clinical Centre of Serbia, Belgrade, and written informed consent was obtained from each patient prior to study engagement.

Clinical neurological evaluation and electrophysiological examination were conducted immediately after the initial presentation to the medical consulting team for oncology patients prior to initiating any therapy.

Clinical neurological evaluation

Neurological examinations were performed repetitively by an experienced physician (S.T.V.) in each case. The presence of neuropathy was clinically defined through the presence of sensory or motor signs, and the reduction or absence of myotatic reflexes without pathological reflexes and symptoms (weakness, sensory disturbances, and burning paresthesia). Clinical evaluation was based on the Neurological Symptom Score and the Neurological Disability Score [13].

After a careful history and neurological examination, patients were further evaluated and the presence of neuropathy eventually confirmed by nerve conduction studies in each case.

Electrophysiological evaluation

Standard nerve conduction studies were performed bilaterally in the upper (the median and ulnar nerves) and lower limbs (the tibial and sural nerves), by standard procedures with surface electrodes for stimulation and recording using a Synergy electromyography machine (Viasys, UK).

Parameters measured included: compound motor action potentials (CMAPs) recorded from the *abductor pollicis* (APB), *abductor digiti minimi* (ADM), *extensor diggitorum brevis* (EDB) and *flexor hallucis brevis* (FHB). Distal and proximal motor latencies, (DML and PML, respectively), motor conduction velocities (MCV), and F-wave latency

were estimated (shortest at each case). The sensory nerve action potentials (SNAPs) were investigated in the median, ulnar, and sural nerves using antidromic recordings from ring electrodes at the 2nd and 5th digits for the median and ulnar nerves, respectively, and toe finger for the sural nerve.

The MCVs were calculated from the following nerve segments: wrist to elbow (median nerve), wrist to below the elbow (ulnar nerve), ankle to popliteal *fossa* (tibial nerve), and ankle to just below the fibular head (peroneal nerve), while sensory conduction velocity (SCV) was calculated for the distal segment, using the antidromic method. The amplitudes of the CMAPs and SNAPs were measured from the baseline to the first negative peak.

Values for the lower limits of normal were defined initially as mean values reduced by 2 standard deviations of normative data for this laboratory, studied by the same method. Subsequently, we have additionally decreased the lower limit of normal of conduction velocities according to reference values [14]. Absolute values of the lower limits of normal for all electrophysiological parameters are summarized in Table 1.

Table 1
Absolute values of the lower limits of the normal and decreased values of electrophysiological parameters (90% and 80% LLN, respectively)

Nerves	MCV* (m/s) or SCV† (m/s)			CMAP* (mV) or SNAP† (μV)		
	LLN	90% LLN	80% LLN	LLN	90% LLN	80% LLN
Motor*						
median/ ulnar	46.0	41.4	36.8	4.0	3.6	3.2
peroneal/ tibial	40.0	36.0	32.0	3.0	2.7	2.2
Sensory†						
median/ ulnar	46.0	41.4	36.8	8.0	7.2	6.4
sural	40.0	36.0	32.0	8.0	7.2	6.4

MCV – motor conduction velocity; CMAP – compound motor action potential; SCV – sensory conduction velocity; SNAP – sensory nerve action potential; LLN – lower limit of normal.

For each nerve examined, we defined typical electrophysiological patterns of abnormalities: a demyelinating [15], an axonal (A), and a combined axonal/demyelinating (A/D) pattern [16]. A demyelinating (D) pattern was defined as when the MCV or SCV ≤ 80% of lower limit of the normal (LLN) and CMAP or SNAP ≥ 80% LLN or MCV or SCV ≤ 70% of LLN and CMAP or SNAP < 80% LLN; an axonal pattern as when the MCV or SCV > 90% LLN and CMAP or SNAP < LLN or MCV or SCV > 80% LLN and CMAP or SNAP < 80% LLN or complete absence of motor or sensory responses after repeated electrical stimulations; a axonal/demyelinating pattern as when the MCV or SCV 80–90% LLN and CMAP or SNAP 80–100% LLN or MCV or SCV below LLN but still above values for demyelinating pattern and CMAP or SNAP in the lower range of normal values (Table 1).

The presence of abnormalities in at least two peripheral nerves was considered as electrophysiological involvement.

Statistical analysis

Descriptive statistics were generated for all variables. The frequency of clinical and electrophysiological parameters was tested using chi-squares (χ^2), and significance was set at the $p < 0.05$ level. The SPSSfor Windows (release 10.0; SPSS, Chicago, IL, USA) was used to perform the statistical analysis.

Results

Demographics and clinical characteristics

During the study period, we have evaluated 105 patients with lung carcinoma. Of the total number of patients, small-cell lung carcinoma, adenocarcinoma, squamocelullar and large-cell carcinoma, according to confirmed histological specimens, accounted for the incidence as shown in Table 2.

Table 2
Patients' demographics and clinical characteristics

Variable	Value
Total sample, n (%)	105 (100)
Sex: male, n (%)	72 (68.6)
Age (years), mean ± SD	63.4 ± 7.8
Tumor type, n (%)	
small-cell carcinoma	32 (30.5)
non-small-cell carcinoma	73 (69.5)
adenocarcinoma	30 (28.6)
squamocelullar carcinoma	39 (37.1)
large-cell carcinoma	4 (3.8)
Neurological symptoms/signs of PNP, n (%)	20 (19.1)

SD – standard deviation; PNP – polyneuropathy.

One thousand and forty-four nerves, consisting of 584 motor, and 447 sensory nerves, on 351 extremities were studied. The mean number (± standard deviation) of studied nerves per patient was 9.85 ± 2.88, of which 5.58 ± 1.69 were motor, and 4.35 ± 1.42 sensory nerves.

Clinical peripheral neuropathy was diagnosed in 20 (19.05%) out of 105 patients, which was confirmed by electrophysiological evaluation. However, nerve conduction studies have identified electrophysiological abnormalities in additional 39 (37.14%). Patients. Overall, electrophysiological abnormalities were present in 59 out of 105 (56.2%) patients.

An axonal, a demyelinating, and a combined axonal/demyelinating patterns were diagnosed in 42/59 (71.2%), 2/59 (3.41%) and 15/59 (25.4%) patients, respectively. However, the patients with described nerve conduction abnormalities did not differ significantly regarding histopathological tumor type, small vs. non-small cell lung cancer (Mantel-Haenszel χ^2, 0.294; $p = 0.86$).

An additional analysis of the above mentioned abnormalities referred to the symmetry of peripheral nerve involvement, showed that 51 out of 59 (86.4%) patients had a symmetrical pattern of damage while only eight (13.6%) had asymmetric nerve lesions.

Considering the entire sample of patients, the distribution of the fiber types (motor vs. sensory) and the pathological mechanism of their damage is shown in Figure 1.

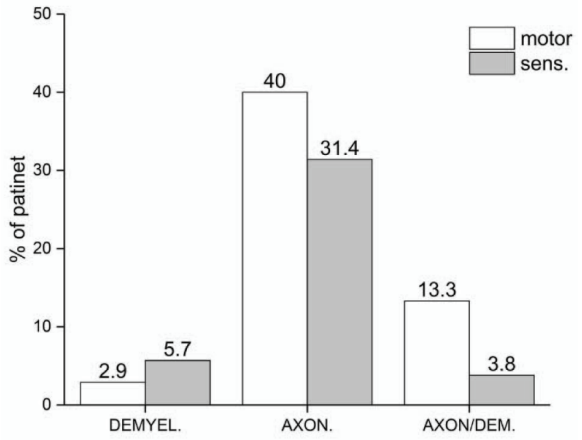

Fig. 1 – Frequency of various forms of peripheral nerve damage in patients with lung carcinoma.

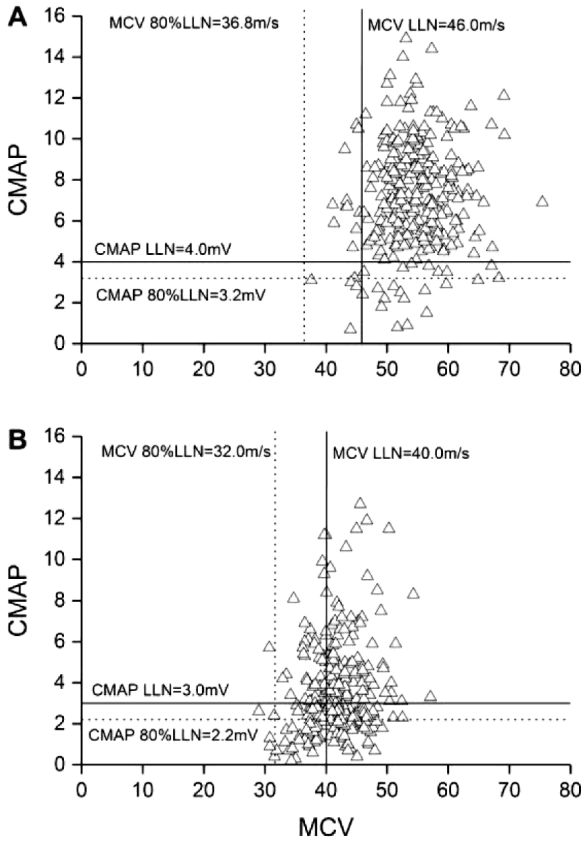

Fig. 2 – Distribution of individual data of motor conduction velocity (MCV) (x-axis) vs. compound motor action potentials (CMAP) (y-axis) in the form of bivariate scattergram for: A) a total of 307 upper limbs motor nerves (*n.medianus* + *n.ulnaris*), and B) 277 lower limbs motor nerves (*n. peroneus* + *n. tibalis*).

Regarding the type of neuropathy, we observed 19/59 (32.2%) of patients with pure motor (predominantly axonal type) neuropathy, 9/59 (15.3%) with sensory neuropathy, and 31/59 (52.5%) of patients with combined sensory-motor neuropathy.

Since the study analyzes a substantial number of peripheral motor and sensory nerves, the results of the distribution of motor and sensory nerve conductions are shown in the form of scattergram separately, as well as for the upper and lower extremities (Figures 2 and 3). These scattergrams show a notable frequency of lower CMAP amplitude (axonal pattern of damage), manifested by a significant grouping of data points in the lower part of the diagram, for motor nerves of the lower extremities, and less evident for sensory nerves of the upper extremities.

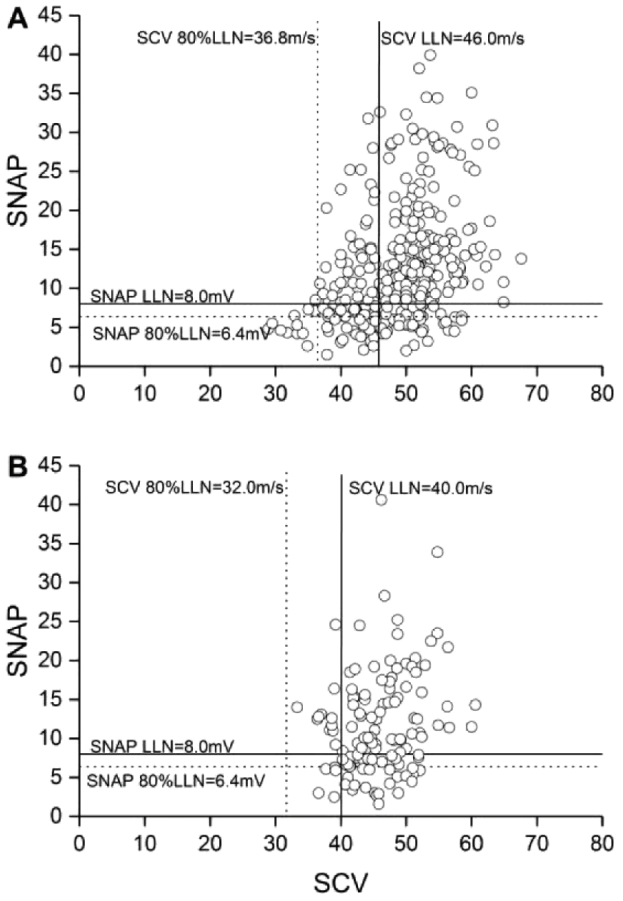

Fig. 3 – Distribution of individual data of sensory conduction velocity (SCV) (x-axis) vs. sensory nerve action potentials (SNAPs) (y-axis) in the form of bivariate scattergram for: A) a total of 324 upper limbs sensory nerves (*n. medianus* + *n. ulnaris*), and B) 123 lower limbs sensory nerves (*n. suralis*).

Discussion

The main finding of this study is the unexpectedly high incidence of clinically manifest neuropathies (19.1%) in a sample of older patients with recently diagnosed and histopathologically confirmed lung carcinoma at a very early stage, before applying any therapeutic modality. Moreover, this

frequency was seen in the category of exclusively lung carcinoma patients, where patients suffering from typical known causes of peripheral neuropathy (e.g., diabetes, alcoholism, chronic renal insufficiency, vitamin deficiencies, thyroid gland dysfunction, paraproteins, and cachexia) were excluded [15, 16]. However, the nerve conduction studies evaluation confirmed neuropathy in these patients but further revealed additional subclinical electrophysiological abnormalities in 39 (37.1%) clinically silent patients. Aggregating clinical and electrophysiological abnormalities in our sample of early-stage lung cancer patients, it was found that 59 out of 105 (56.2%) od patients showed some degree of peripheral nerves damage. The most frequent pathophysiological pattern, within the group of patients with confirmed nerve damage, was the axonal pathology for sensory (31.4%) as well as for motor (40%) nerves.

Recent studies of lung carcinoma patients point to an increasing incidence of this disease and high mortality, where the non-small-cell type of lung cancer (NSCLC) is seen in up to 85%–90% of patients, while the frequency of small-cell lung cancer (SCLC) is decreasing [17]. Alberg and Samet [18] underlined that the epidemics of lung cancer is positively correlated to cigarette smoking and that the most common histological subtypes are the squamocellular (SCC) lung cancer and SCLC. Newer studies have repeatedly shown a predominance of adenocarcinomas. Our study confirms these results. The lung adenocarcinoma was found in almost 29% of our patients. The reducing frequency of SCLC in patients who have lung cancer is explained by the reduced number of smokers and improvements of the cigarette filters [19]. Our study showed still high frequency of SCLC cancer, evident in almost 31% of our patients. A recent meta-analysis of studies exploring the influence of cigarette smoking on histological subtypes of lung cancer has shown a stronger correlation with SCLC and SCC. Considering the description of the smoking-related microcellular carcinoma, we can assume this fraction is so large in our patients because all our patients have been smoking for more than 30 years.

The distribution by gender of our patients confirmed the dominance of the male sex, with around 69% of males among the patients, although some authors report an increasing incidence of women suffering from lung carcinomas, which is leading to almost equal frequencies of male and female patients [19].

The underlying mechanisms of peripheral nerve damage in lung carcinoma patients are seen as consequences of cytotoxic chemotherapy, peripheral nerve or roots infiltration, metabolic or nutritive deficiencies. When all previously mentioned factors are excluded, and with a confirmation of the immune mechanisms activation, the neuropathy can be considered as paraneoplastic (PNN) [8]. The mechanism of damage has been connected to onconeural antibodies together with onconeural antigen-specific T-lymphocytes, although the absence of known onconeural antibodies in patients with so-called "classic" clinical presentations does not exclude the possibility of PNN [3]. These are often complicated forms of neuropathies which point to the diagnosis of the underlying disease, and sometimes cause a high level of disability regardless of cancer as the primary disease [20].

Nevertheless, the causal relationships of neuropathies, above all of sensorimotor type, in this population of patients, add controversy, considering we are discussing an elderly population (in our study 63.5 was the mean age), with many potential underlying causes of peripheral nerve damage, such as: metabolical (diabetes, chronic kidney disease), nutritive or iatrogenic (cytotoxic, e.g. paclitaxel, cisplatin, vinca alkaloids, but also non-cytotoxic pharmaceuticals, e.g. antimicrobial agents, cardiovascular medication, etc.). The etiology of these neuropathies in cancer patients can be considered multifactorial, which includes the mentioned metabolical and nutritive deficiencies, but also unrecognized factors which appear in later stages of the primary disease [21].

In our sample, the patients were meticulously selected, at the very beginning of the disease, excluding all patients with cachexy, recognizable nutritive deficits, or those with risk factors for most common metabolical, nutritive, or immunologically mediated neuropathies typical for this age group. Nevertheless, after a detailed examination and neurophysiological evaluation of each patient we have found an unexpectedly high percentage of, above all, electrophysiological, but also clinical abnormalities. Finally, the pattern of damage which appeared as the dominant one in our sample was the distal sensorimotor neuropathy, with mostly axonal damage.

In case we tried to compare our results with similar studies, we came across a relatively small number of studies exploring the etiology of peripheral neuropathies in elderly patients, but not suffering from lung cancer [22, 23]. It is important to note that, based on modern guides for diagnosis of neuropathies, with increasing accuracy over the years, more than a hundred of different causes have been identified [24]. Nevertheless, even after applying all of the proposed criteria, for a certain number of patients (around one fourth) it is impossible to identify the cause of neuropathy, and this is considered a chronic idiopathic axonal neuropathy (CIAN) [25]. In general, the pathophysiological mechanism underlying this type of neuropathy is axonal degeneration, which is, as a rule, irreversible, and consequently, a permanent loss of nerve fibers occurs. However, in addition to the increased incidence of axonal lesions on our sample of early-stage lung cancer patients and the fact that older people are more likely to determine CIAN, it is interesting to consider whether there is any relationship between the axonal type of neuropathy and lung disease in general. The possibility that a chronic obstructive pulmonary disease, based on presumed hypoxia, may lead to the axonal pathology of the peripheral nerves has been refuted so far [26]. In considering the CIAN etiology, special interest is currently focused on disturbed peripheral nerve *vasa nervorum* microcirculation, which is associated with the metabolic risk factors, but also the diseases of peripheral circulation of the atherosclerotic type [27]. As this series of patients, according to our knowledge, is the first of its kind on the patient population in our country, it is therefore not possible to link the observed neuropathic changes to lung carcinoma, as an etiological factor. In our view, future studies of similar interest should define the presence of chronic risk factors for peripheral microcirculation disorders, in particular with laboratory values (e.g. blood glucose) within the upper limits of normal.

Conclusion

Patients suffering from lung cancer at the moment of initial presentation show a high frequency of clinically apparent, as well as clinically silent neuropathies. Based on the characteristics, these neuropathies, manifesting themselves symmetrically in distal parts of the extremities, with most common electrophysiological pattern of axonal damage, suggest a common existence of a chronic axonal idiopathic neuropathy. Knowing of the possibility of a high frequency of mild subclinical neuropathies, seen mostly in the elderly population, suggests particular attention is needed when evaluating lung cancer patients in early diagnostic phase, to confirm or exclude this possibility. The most important implications of these results are on the analysis of the frequency and probability of clinically manifest neuropathies appearing during the treatment of the primary disease, e.g., using chemotherapeutic protocols.

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The increased main branch to stent diameter ratio is associated with side branch decreased coronary flow in "true" coronary artery bifurcations treated by "provisional" stenting – a two-dimensional quantitative coronary angiography study

Uvećanje odnosa dijametra glavne grane i veličine stenta povezano je sa smanjenim protokom u bočnoj grani kod "pravih" račvi koronarnih arterija lečenih "provizornom" implantacijom stenta – studija sa dvodimenzionalnom kvantitativnom koronarografijom

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Abstract

Background/Aim. Percutaneous coronary interventions (PCI) in bifurcation lesions having more than 50% stenosis of both the main branch (MB) and the side branch (SB) remain challenging. Measurements of the vessel diameters and angles using quantitative coronary angiography (QCA) software have been used in evaluating PCI outcomes. We investigated potential effects of provisional stenting of the MB on SB coronary blood flow by determining quantitative vessel parameters in "true" non-left main coronary bifurcation lesions using conventional two-dimensional QCA. **Methods.** The study was prospective and conducted in a high-volume university PCI center. Study included patients with "true" native coronary artery bifurcations (Medina 1.0.1; 0.1.1; 1.1.1) treated with "provisional" stenting of the MB. Patients were excluded from the study if having left ventricular ejection fraction of less than 30%, having renal failure with creatinine clearance below 30 mL/kg/m^2 or bifurcation lesions within the culprit artery causing myocardial infarction, grafted surgically or previously treated by PCI. QCA analysis included measurements of reference vessel diameters (RVD), diameter stenosis (DS) and bifurcation angles. **Results.** The study included 70 patients with 72 "true" non-left main bifurcations. Most of the bifurcations were located in the left anterior descending (LAD) – diagonal (Dg) territory. Compromise of the SB ostium defined as thrombolysis in myocardial infarction (TIMI) < 3 coronary flow occurred in 17/72 (23.6%) bifurcations. It was treated by either balloon angioplasty only of the SB ostium (9/17, 52.9%) or stent implantation [8/17 (47.1%)]. In a logistic regression analysis, including previously recognized predictors of SB compromise (bifurcation's angles, RVD, DS and ratio of MB RVD to stent diameter ratio), only MB RVD to stent diameter ratio after PCI remained independent predictor of SB coronary flow compromise after stent implantation in the MB [OR 2.758 (95% CI 1.298–5.862); $p = 0.008$]. **Conclusions.** It appears that SB decreased coronary blood flow after "provisional" stenting in "true" non-left main bifurcations is associated with greater MB to stent diameter ratio.

Key words:
percutaneous coronary intervention; stents; blood circulation; coronary angiography.

Apstrakt

Uvod/Cilj. Perkutane koronarne intervencije (PKI) lezija na račvi sa suženjem većim od 50% na glavnoj grani (GG) predstavljaju izazov. Procena dijametara i uglova primenom programa kvantitativne koronarografije (KK) korišćena je u proceni ishoda PKI. Istraživali smo potencijalne efekte „provizorne" implantacije stenta u GG na protok u bočnoj grani (BG) određivanjem kvantitativnih parametara krvnog suda kod „pravih" račvi koje ne zahvataju glavno stablo leve

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koronarne arterije, primenom 2D KK programa. **Metode.** Prospektivna studija, sprovedena u univerzitetskom centru sa velikim brojem PKI, uključila je bolesnike sa „pravim" račvama nativnih koronarnih arterija (Medina 1.0.1, 0.1.1, 1.1.1) lečene „provizornom" implantacijom stenta u GG. U studiju nisu bili uključeni bolesnici sa ejekcionom frakcijom (EF) manjom od 30%, klirensom kreatinina manjim od 30 mL/kg/m^2 ili lezijom na račvi odgovornoj za infarkt miokarda, hirurški revaskularizovanoj ili prethodno lečenoj primenom PKI. KK analizom mereni su referentni dijametri (RD) GG i BG, stepen dijametar suženja (DS) i uglovi bifurkacija. **Rezultati.** Studija je uključila 70 bolesnika sa 72 račve. Većina račvi nalazila se u slivu prednje descedentne grane (PDA) – dijagonalna arterija (Dga). Kompromitacija ostijuma BG definisana kao smanjenje koronarnog protoka TIMI < 3 nastala je kod 17/72 (23,6%) račve. Tretirana je angioplastikom ostijuma BG [9/17 (52,9%)] ili implantacijom stenta [8/17 (47,1%)]. U logističkoj regresionoj analizi koja je uključila prethodno poznate prediktore kompromitacije BG (uglovi bifurkacije, RD, DSi odnos GG RD prema dijametru stenta) samo je odnos RD GG prema dijametru stenta bio nezavisni prediktor smanjenje protoka u GG nakon implantacije stenta [OR 2,758 (95% CI 1,298 – 5,862); p = 0,008]. **Zaključak.** Sniženje koronarnog protoka u bočnoj grani nakon „provizorne" implantacije stenta u glavnu granu, kod „pravih" račvi koronarnih arterija, povezano je sa uvećanim odnosom dijametra glavne grane prema dijametru stenta na osnovu dvodimenzionalne KK.

Ključne reči:
perkutana koronarna intervencija; stentovi; krv, cirkulacija; angiografija koronarnih arterija.

Introduction

Percutaneous coronary interventions (PCI) on bifurcation lesions comprise about 15–20% of all PCI procedures and are associated with increased incidence of complications and stent thrombosis [1, 2]. "Provisional" stenting is a recommended strategy for most bifurcations, since it provides favorable angiographic and clinical outcomes [3, 4]. Treatment of bifurcation lesions having more than 50% stenosis of both the main branch (MB) and side branch (SB), remains difficult. These, so called, "true" bifurcations are often underrepresented in the clinical trials and their treatment remains challenging. It is conceivable that "provisional" stenting of the MB in "true" bifurcation can further compromise already stenosed SB ostium causing ischemia by "plaque" or "carina shift" [3, 5]. Since visual estimation has been deemed insufficient, two dimensional (2D) quantitative coronary angiography (QCA) is increasingly used in practice and clinical research to assess severity of obstructive coronary artery disease and the efficacy of the PCI [6, 7]. Measurements of the vessel diameters and angles using QCA software before and after the PCI on bifurcations have been used in evaluating outcomes of the interventions and also the mechanism of SB compromise [4, 5]. Since division of coronary arteries at sites of bifurcations follows fractal law and the curvature of the heart causes foreshortening of the vessels, conventional 2D QCA software may not be optimal for the analysis of coronary artery bifurcations [8, 9]. Recently, dedicated packages for bifurcations and three dimensional (3D) QCA software have been developed to further improve bifurcation analysis [10].

We investigated the mechanism of side branch decreased coronary blood flow after "provisional" stenting of the main branch by determining quantitative vessel parameters of both, main and side branch in "true" non-left main coronary bifurcation lesions using conventional two-dimensional quantitative coronary angiography.

Methods

The study was prospective and conducted in a high-volume university PCI center. Study included patients with "true" native coronary artery bifurcations (Medina 1.0.1; 0.1.1; 1.1.1) [11] with > 50% visually estimated stenosis in both MB and SB, with SB diameter greater than 2 mm and SB stenosis extending not more then 5 mm from the ostium. Patients were scheduled to undergo PCI based on clinical findings of stable angina, silent ischemia and/or proven coronary ischemia on functional testing. The patients signed an informed consent form prior to any of the study procedures. The study was approved by the institutional Ethics committee and was done in accordance with Helsinki declaration. This study was the part of project that evaluated "true" coronary bifurcations scheduled for PCI using multimodality imaging with multislice computed tomography coronary angiography, intravascular ultrasound (IVUS) and invasive coronary angiography (study protocol was registered at www.clinicaltrials.gov- NCT01943643).

Patients were excluded from the study if having depressed left ventricular ejection fraction (LVEF) of less than 30% or suffering from renal failure with estimated glomerular filtration rate (GFR) of less than 30 mL/min/m^2. Patients with GFR between 30 to 60 mL/min were adequately hydrated before PCI. Patients with bifurcation lesions within the culprit artery causing myocardial infarction, grafted surgically or previously treated by PCI, were not considered for the study. Patients having medical condition that can be a contraindication for PCI and/or an allergy to aspirin, clopidogrel, ticagrelor or contrast agent were also excluded from the study.

Initial strategy for PCI was "provisional" stenting of the MB. Vascular access, guiding catheters and coronary wires were chosen by the operators. Heparin in doses of 80–100 IU/kg was used as periprocedural anticoagulation. After placing the guidewires in the MB and SB lesion, the MB was predilated. After adequate predilation, a second generation drug eluting stent (DES) was placed in the MB across the SB, so that its diameter was chosen by the operator based on the visual estimate of the distal MB vessel size and in line with the fractal law of bifurcations [8]. After stenting, proximal optimization technique (POT) of the stent in the MB was performed using high pressure inflation with a short non-compliant balloon catheter, 0.25–0.5 mm larger than the di-

ameter of the stent implanted. After POT and intracoronary nitroglycerin administration, coronary angiograms in two orthogonal projections were done. If coronary blood flow in SB was normal with thrombolysis in myocardial infarction (TIMI) flow grade 3, the procedure was finished. If the flow in the SB was less than TIMI 3, procedure was continued with guidewire exchange, preferably with a third guidewire inserted through the distal strut in the SB. After predilatation of the SB ostium, kissing inflation was done using adequately sized noncompliant balloon catheters in the MB and SB, up to 6–8 atm. After control coronary angiogram, if the SB TIMI flow remained less than 3 or there was an ostial dissection of the SB, another second generation DES was implanted in the SB, using the technique chosen by the operator. The preferred technique was T and protrusion (TAP).

Coronary angiograms before and after PCI were analyzed by experienced interventional cardiologist, not aware of patient clinical and procedural data. Analysis was performed offline using commercially available software package for 2D QCA installed on multimodality workstation Leonardo (Siemens, Erlangen, Germany). The same projections were used for pre- and post-intervention image analyses. End-diastolic frames were selected for interpretation to avoid foreshortening and overlapping of the coronary arteries. The manual calibration was done according to the guiding catheter size. Using automated border detection software, analysis of two vessels was performed, one starting from the proximal MB to distal MB, and another one starting from the proximal MB and towards the SB (Figure 1). The manual corrections were applied in order to include atherosclerotic plaque in MB and at the ostium of the SB that may have been missed by automated detection.

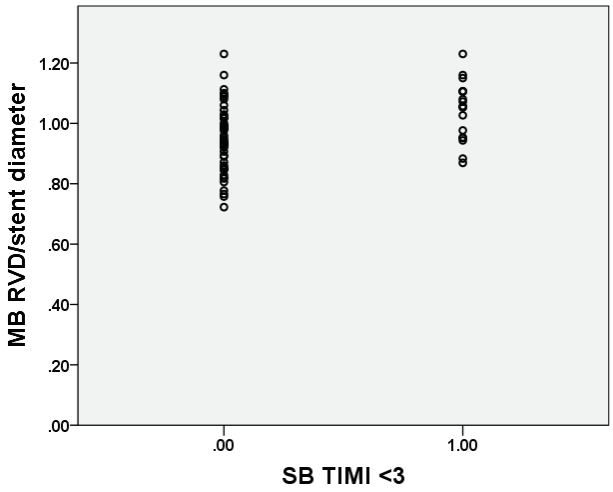

Fig. 1 – Native coronary artery bifurcation LAD–Dg, with the drawing depicting the method of vessel tracing for QCA analysis and acquired measurements. LAD – left anterior descending; Dg – diagonal branch; QCA – quantitative coronary angiography; RVD – reference vessel diameter; TIMI – thrombolysis in myocardial infarction.

The analysis included measurements of the proximal and distal reference diameters in the MB defined as the least affected segment by atherosclerosis up to 10 mm from the bifurcation plaque. Measurements included SB reference diameters defined as the least diseased segment up to 10 mm distal from the SB ostium, maximum diameter stenosis (DS), area stenosis (AS) of the MB and SB ostium, as well as all bifurcation angles (proximal MB – SB, distal MB – SB, proximal MB – distal MB).

The reproducibility of the QCA parameters measured MB and SB RVD before and after PCI and bifurcation angle B before and after PCI were measured by two experienced observers and twice by each observer in 20 randomly selected patients. Interobserver coefficients of variation for measuring MB and SB RVD before and after PCI and bifurcation angle B before and after PCI were 5%, 7%, 5%, 9%, 5% and 6%, respectively. Intraobserver coefficients of variation for repeated same measurements were 4%, 6%, 5%, 8%, 7% and 3%, respectively.

After the PCI, the patients with uneventful clinical course stayed in the hospital for 24 h. Blood samples for troponin I, creatine kinase (CK) and CK-MB were collected at 12 and 24 h after the procedure, and for C-reactive protein (CRP) after 24h. Complications of the interventions were documented in the patients' study file.

Statistical analysis

Continuous data were summarized as the means ± standard deviation (sd) or as median value and interquartile range (IQR) depending on distribution. Categorical data were summarized as counts and percentages. Unpaired *t*-test was used for comparing the continuous variables if their distribution was normal and Mann-Whitney *U* test was used for the continuous variables that did not have normal distribution. The chi-squared (χ^2) test and Fisher's exact test were used for categorical variables. The effect of patient's 2D QCA characteristics on endpoint of SB decrease in coronary flow bellow TIMI 3 were explored using multivariate logistic regression and their impact were expressed as odds ratios (OR) with 95% confidence intervals (CI). A set of variables was selected prospectively, and a stepwise selection process was used to determine independent predictors of SB compromise in a multivariate analysis model. The stepwise selection iteratively selected the most significant variable with multivariate p-value < 0.25, to start the model. At each step, another significant variable was added and after running the model, a check was performed to remove the variable with a multivariate p-value > 0.10. This was repeated with the complete set of variables until no more variables could be entered and no variables could be dropped. The p value of $p < 0.05$ was considered to be statistically significant. All statistical analyses were performed using PASW Statistics 18.0 statistical software (SPSS Inc., Chicago, Illinois, USA).

Results

The study included 70 patients with 72 "true" non-left main bifurcations. Patients included in the study had high incidence of hypertension, dyslipidemia and previous myocar-

dial infarction. Also, they had preserved left ventricular ejection fraction and renal function. The clinical characteristics were similar in the groups of patients with or without disturbance of SB coronary flow (Table 1).

Majority of the patients included in the study had two-vessel disease [1 vessel – 17/70 (24.3%), 2 vessel – 43/70 (61.4%) and 3 vessel disease 10/70 (14.3%)]. Most of the bifurcations were located in the left anterior descending (LAD) – diagonal (D) territory in both study groups. Data regarding the distribution of bifurcations and stenosis according to Medina classification were presented in Table 2.

All patients underwent PCI using radial access and 6F guiding catheter. After successful stent implantation and POT, SB decreased coronary flow TIMI < 3 occurred in 17/72 (23.6%) bifurcations. It was treated by either balloon angioplasty only of the SB ostium (9/17, 52.9%) followed by POT (POT – side – POT) or stent implantation (8/17, 47.1%) followed by kissing-balloon inflation and POT (Table 2). All interventions were deemed successful with no significant residual stenosis and final TIMI 3 flow in the MB.

Two-dimensional QCA revealed that only MB reference vessel diameter and residual diameter stenosis were significantly different in the group of bifurcations with SB TIMI < 3 after "provisional" stenting. There was an increase in MB RVD in the group of patients where SB compromise occurred. Also, derived parameter – MB RVD after PCI to implanted MB stent diameter ratio – was significantly greater in the SB compromise group (Table 3, Figure 2).

Table 1

Clinical characteristics of the patients in the study groups

Variable	SB TIMI = 3 (n = 55)	SB TIMI < 3 (n = 17)	*p*
Age (years), mean ± sd	58 ± 8	61 ± 9	0.242
Male gender, %	72.5	72.2	1.000
Heredity, %	38.1	47.1	0.409
Smoking, %	43.6	29.4	0.177
Hypertension, %	87.2	88.2	0.600
Dyslipidemia, %	63.6	83.3	0.358
Diabetes mellitus (DM), %	16.4	23.5	0.730
Insulin dependent DM, %	7.2	5.9	0.567
PAD, %	3.9	1.1	0.277
Previous MI, %	58.8	44.4	0.409
Previous CVA, %	3.9	0.0	1.000
Previous PCI, %	54.9	55.5	1.000
Previous CABG, %	0.0	0.0	
BMI (kg/m^2), median (IQR)	27.4 (25.7–29.9)	27.9 (26.4–34.3)	0.214
LVEF (%), median (IQR)	50 (40–60)	57 (48–60)	0.184
eGFR (mL/min/m^2), median (IQR)	82 (67–94)	96 (73–112)	0.050

SB – side branch; PAD – peripheral arterial disease; MI – myocardial infarction; CVA – cerebrovascular accident; PCI – percutaneous coronary intervention; CABG – coronary artery bypass grafting; BMI – body mass index; LVEF – left ventricular ejection fraction; eGFR – estimated glomerular filtration rate; sd – standard deviation; IQR – interquartile range.

Table 2

Angiographic and percutaneous coronary intervention characteristics of bifurcations

Bifurcation characteristics	SB TIMI = 3 (n = 55)	SB TIMI < 3 (n = 17)	*p*
Location, n (%)			
LAD–Dg	38 (69.1)	12 (70.6)	0.576
Cx–OM	13 (23.6)	4 (23.5)	0.594
RCA PD–PL	4 (7.2)	1 (5.9)	0.723
Medina classification, n (%)			0.444
1.0.1	14 (25.5)	2 (11.7)	
0.1.1	18 (32.7)	6 (35.3)	
1.1.1	23 (41.8)	9 (52.9)	
Stent diameter in MB (mm), mean ± sd	3.1 ± 0.4	3.0 ± 0.2	0.243
Stent length in MB (mm), mean ± sd	25.5 ± 6.4	26.4 ± 4.3	0.616
Maximum stent inflation (atm), mean ± sd	14.0 ± 1.43	13.8 ± 1.5	0.484
POT balloon diameter (mm), mean ± sd	3.6 ± 0.4	3.5 ± 0.4	0.504
POT balloon length (mm), mean ± sd	8.2 ± 2.6	8.6 ± 2.2	0.590
POT balloon maximum inflation (atm), mean ± sd	17.7 ± 1.9	17.2 ± 1.8	0.345

Cx – circumflex; Dg – diagonal; LAD – left anterior descending; MB – main branch; OM – obtuse marginal; PD – posterior descending; PL – posterolateral; POT – proximal optimization treatment; RCA – right coronary artery; TIMI – thrombolysis in myocardial infarction; sd – standard deviation.

Table 3

Quantitative coronary angiography characteristics in bifurcations with or without side branch decreased flow

QCA characteristics	SB TIMI = 3 mean ± sd	SB TIMI < 3 mean ± sd	*p*-value
MB RVD before PCI (mm)	2.8 ± 0.2	2.9 ± 0.4	0.373
MB DS before PCI (%)	64 ± 8	66 ± 9	0.425
SB RVD before PCI (mm)	2.4 ± 0.2	2.5 ± 0.3	0.106
SB DS before PCI (%)	59 ± 8	63 ± 8	0.082
MB RVD post PCI (mm)	2.9 ± 0.3	3.2 ± 0.4	0.008
Delta MB RVD (mm)	0.10 ± 0.26	0.27 ± 0.31	0.030
MBDS post PCI (%)	13 ± 9	21 ± 12	0.009
SB RVD post PCI (mm)	2.6 ± 0.3	2.7 ± 0.3	0.507
SB DS post PCI (%)	62 ± 16	64 ± 26	0.656
Angle B before PCI	58 ± 17	59 ± 20	0.886
Angle B after PCI	56 ± 18	58 ± 18	0.825
MB RVD post PCI to SD ratio	0.95 ± 0.11	1.05 ± 0.09	0.001

Angle B – angle between distal MB and SB; Delta – change in main branch reference vessel diameter; DS – diameter stenosis; MB – main branch; PCI – percutaneous coronary intervention; RVD – reference vessel diameter; SB – side branch; SD – stent diameter; TIMI – thrombolysis in myocardial infarction; sd – standard deviation.

Table 4

Univariate and multivariate predictors of side branch compromise (TIMI < 3)

Predictors	Univariate		Multivariate	
	OR (95% CI)	*p*–value	OR (95% CI)	*p*–value
Bifurcation angle B	1.005 (0.975–1.036)	0.734	–	–
RVD MB before PCI	2.525 (0.331–19.288)	0.372	–	–
RVD SB before PCI	5.918 (0.654–53.539)	0.114	–	–
MB DS before PCI	1.028 (0.961–1.099)	0.420	–	–
SB DS before PCI	1.067 (0.991–1.150)	0.086	1.074 (0.987–1.169)	0.097
MB DS after PCI	1.078 (1.015–1.145)	0.015	1.038 (0.958–1.126)	0.359
SB DS after PCI	1.007 (0.977–1.038)	0.651	–	–
MB RVD after PCI/SD	2.695 (1.424–5.099)	0.002	2.758 (1.298–5.862)	0.008
Delta MB RVD	8.352 (1.156– 60.332)	0.035	2.268 (0.187–27.570)	0.521

Angle B – angle between distal MB and SB; Delta – change in main branch reference vessel diameter; DS – diameter stenosis; MB – main branch; PCI – percutaneous coronary intervention; RVD – reference vessel diameter; SB – side branch; SD – stent diameter; TIMI – thrombolysis in myocardial infarction; OR – odds ratio; CI – confidence interval.

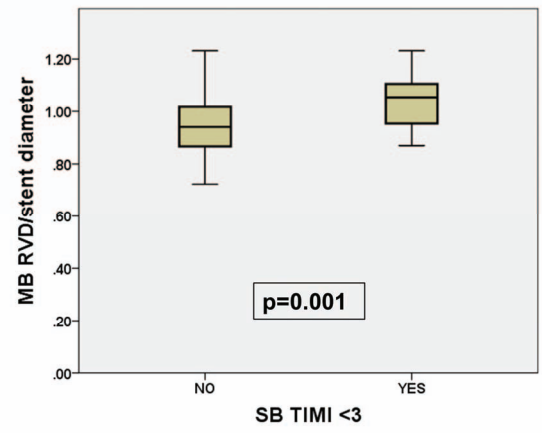

Fig. 2 – Diagram presents the relationship between occurrence of SB compromise defined as TIMI flow < 3 on the X-axis, and a derived variable representing the median value and interquartile range of ratio between the MB reference vessel diameter and stent diameter after stent implantation on the Y-axis.
MB – main branch; SB – side branch; RVD – reference vessel diameter; TIMI – thrombolysis in myocardial infarction.

In a multivariate logistic regression analysis, that included previously recognized predictors of SB compromise (bifurcation angle between distal MB and SB, RVD of MB and SB before and after PCI, DS of MB and SB before and after PCI, change in MB RVD after PCI and ratio of MB RVD/stent diameter), only MB RVD to stent diameter ratio measured after PCI remained independent predictor of SB decreased coronary blood flow after stent implantation in the MB (Table 4).

Discussion

Our study demonstrated that in "true" non-left main native coronary artery bifurcation treated by "provisional" stenting strategy, increase in the MB diameter relative to the diameter of the implanted stent, was independently associated with SB decreased coronary blood flow. To our knowledge, this was not been previously described and it may serve as a measurement of MB vessel enlargement relative to implanted stent diameter. It perhaps may be considered as a surrogate value to evaluate the occurrence of "carina" shift

as a potential mechanism of the SB compromise. Also, despite its inherent limitations, we showed that conventional 2D QCA still can be useful in determining vessel diameters and angles in complex bifurcations and for guiding PCIs.

Atherosclerotic plaques have specific pattern of development in coronary artery bifurcations. They tend to develop in the areas of low endothelial shear stress (ESS), at the lateral walls of bifurcations. Contrary, areas with high ESS, like bifurcation's carina, tend to be spared in the initial development of bifurcation lesion [12, 13]. We analyzed bifurcations with already advanced atherosclerosis, with large plaque volumes, manifested as significant luminal narrowing of both the MB and SB (Table 3). The SB TIMI < 3 occurred in almost one fifth of the patients. This is probably the consequence of advanced atherosclerotic disease in both the MB and ostium of SB, leading to SB decreased coronary flow after stenting [14].

In our study we used commercially available 2D QCA software. This software ignores the fact that coronary artery bifurcations divide according to the fractal law and that coronary arteries follow three-dimensional curvature of the heart. These could lead to erroneous interpretation of the vessel size and the severity of the atherosclerotic lesions, especially at the points of SB origin [10]. Of note, dedicated bifurcation software has been developed, that appreciated the true geometry of the bifurcation and yielded more precise and reproducible bifurcation analysis [15].

The patients in the SB compromise group had greater DS of the SB ostium before the intervention that was no longer true after "provisional" stenting of the MB. Dou et al. [16] incorporated SB ostial stenosis into scoring system to predict SB occlusion after stenting. The higher grade of SB ostial stenosis produced higher score and subsequently higher risk of SB compromise. Similarly, a study using OCT analysis of the bifurcation by Kini et al. [17], demonstrated that SB stenosis is an independent predictor of SB occlusion. In our patients, preprocedural SB DS was not independently associated with decreased coronary flow in the SB. The SB DS before PCI was associated with SB compromise in univariate analysis but this relationship was not confirmed in multivariate model. This may be explained by the fact that we used the 2D QCA software that tends to overestimate significance of SB ostial stenosis and also by the fact that the mechanism of "carina shift" may be predominantly responsible for SB coronary flow compromise [5, 9, 10, 18].

Surprisingly, in the multivariate analysis, the angle between distal MB and SB was not associated with SB decreased coronary blood flow. This finding corroborates with some studies that found that either wide or narrow bifurcation angles were not associated with SB compromise [19, 20]. It is important to notice that our analysis of bifurcation angles was done in two dimensions and did not include assessment of an "inflow" angle of the bifurcation – the angle between the proximal MB and "bifurcation plane", where distal MB and SB are situated. This may have resulted in lack of evidence of association between bifurcation angles, as we calculated them, and the SB flow bellow TIMI 3 [21]. The influence of the bifurcation angle on the fate of the SB after stenting should probably be studied by appreciating the three dimensional angulation of the coronary arteries and the curvature of the heart.

We showed that only ratio of the MB RVD to stent diameter after PCI was independently associated with decreased SB flow. This relationship perhaps may be the consequence of predominant "carina shift" effect. This phenomenon is the outcome of the stent implantation in the MB that causes movement of the carinal structure of the bifurcation towards the SB leading to its obstruction. Although "plaque shift" can still be the factor in SB compromise, the current paradigm regarding SB compromise after MB stenting is that in most of the cases the SB ostium is jeopardized by "carina shift". This may be further augmented by already present significant ostial disease of the SB, resulting in critical stenosis of the SB ostium after MB stenting [18, 22].

The studies using IVUS contributed significantly to the body of knowledge regarding SB compromise and "carina shift". The study by Xu et al. [5], examined by IVUS both the MB and SB before and after PCI and found that after intervention diameters and volumes of the MB increase, while the opposite occurs at the ostium of the SB. Also, it was demonstrated that SB compromise is frequently caused by vessel volume decrease which is a surrogate of "carina shift", instead of vessel plaque increase, which is a surrogate of "plaque shift". In the study by Koo et al. [18] was also shown that there is a strong correlation between SB ostial compromise and increase in diameters and volumes of distal MB, suggesting that excessive stent expansion can contribute to "carina shift" and increase in ostial SB stenosis. The variable in our study that was independently associated with SB decreased TIMI flow (RVD of the MB to stent diameter ratio), contains the value of stent diameter as an important determinant of vessel enlargement after PCI and possible "carina" shift. Morphology of the carina, evaluated by IVUS, can influence SB compromise in bifurcations without SB ostial disease, as demonstrated by Suárez de Lezo et al. [23], emphasizing the importance of "carina shift" as mechanism causing SB compromise. SB compromise can be further aggravated by stent expansion that exceeds diameter of the main vessel. Our data confirmed that greater expansion of the vessel relative to the stent diameter after "provisional" stenting is independently associated with SB ostial compromise.

Study limitation

In the study we used commercially available 2D QCA software that ignores the laws of bifurcations' division and three-dimensional curvature of the heart. All this can lead to inaccurate measurements of the vessel's dimensions and angles. Also, the variable that was identified as an independent predictor of the SB decreased coronary flow contains the MB dimensions that are measured after the intervention.

Clinical implications

Adequate estimate of the vessel size can be very challenging especially in complex bifurcations and lead to com-

plications of the interventions. Since careful selection of the stent size according to vessel diameter is crucial in provisional stenting of the MB, the results of our study favour the routine use of any available QCA software in planning the procedure.

Conclusion

SB decreased coronary blood flow after "provisional" stenting in "true" non-left main bifurcations is associated with greater main vessel to stent diameter ratio. Two-dimensional QCA, with its known limitations, can still be useful tool in planning complex bifurcation PCI procedures.

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Impact of active fluid management on cardiac hemodynamics and mechanics in patients on maintenance hemodialysis

Uticaj aktivne kontrole volemije na srčanu hemodinamiku i mehaniku kod bolesnika na hroničnom lečenju hemodijalizom

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Abstract

Background/Aim. Overhydration (OH) and shortcomings of clinical assessment of so called „dry weight" in hemodialysis (HD) patients are well known risk factors for high cardiovascular morbidity and mortality in this population. The purpose of this prospective randomized study was to investigate possible benefits of the active fluid management (AFM) guided by bioimpedance spectroscopy (BIS) on cardiac morphology, mechanics and function in chronic hemodialysis patients. **Methods.** The study lasted 9 months and 83 BIS naive patients were enrolled. Cardiac structural and functional characteristics were obtained using two dimensional Doppler echocardiography and global strains by speckle tracking modality. In addition, cardiac markers were measured. **Results.** Seventy three patients completed the study (38 in the active – AFM group and 35 in the control group). At the end of the study, the main structural change in the active group of patients was reduction of left ventricular mass index (from 62.81 ± 19.74 g/m$^{2.7}$ to 57.74 ± 16.87g/m$^{2.7}$; $p = 0.007$), while main functional improvements in this group were better left ventricular ejection fraction (LVEF; from 41.27 ± 9.26% to 43.95 ± 8.84%; $p = 0.006$) and fractional shortening (FS; 27.86 ± 5.94% to 29.86 ± 5.83%; $p = 0.056$) in accordance with improvement of radial left ventricular (LV) mechanics detected by higher global radial strain (GRS) (18.56 ± 10.24% to 21.79 ± 12.16%; $p = 0.014$). The diastolic function of patients in the control group worsened significantly, assessed as ratio of Doppler velocity of early diastolic filling of left ventricle – E, and average velocity of tissue Doppler measured at lateral part of the mitral annulus (e' lateral; E/e' lateral ratio 10.59 ± 5.00 to 11.12 ± 4.06; $p = 0.036$) and consecutively the right ventricular systolic pressure (RVSP) estimated indirectly by echocardiography: from 34.84 ± 10.18 mmHg to 38.76 ± 8.34 mmHg; $p = 0.028$. These functional changes were in correlation with significantly higher levels of N-terminal prohormone brain natriuretic peptide (NT-proBNP) in this group of patients [median and interquartile range (IQR): 5810.0 pg/mL (3339.0–15627.0 pg/mL) to 8024.0 pg/mL (4433.0–17467.0 pg/mL; $p = 0.038$)]. The improvement in the LV structure and function in the active group correlated with better relative overhydration (ROH) management in this group – the proportion of "critically" overhydrated patients decreased from 45% at the start to 24% at the end of study ($p = 0.003$). At the end of the study, there were 49% of post-dialysis "critically" dehydrated patients in the control group. Proportion of anuric patients increased only in the control group (63% to 77%; $p = 0.063$). **Conclusion.** Active fluid management, guided by bioimpedance spectroscopy had positive impact on cardiac hemodynamics and mechanics in our study patients and could improve clinical decisions regarding their optimal weight and further clinical course. Further data from well designed studies are needed urgently.

Key words:
renal dialysis; ventricular function, left; echocardiography, doppler; biolectric impedance; biomarkers.

Apstrakt

Uvod/Cilj. Hipervolemija i nedostaci kliničkog procenjivanja tzv. „suve težine" kod bolesnika na lečenju hemodijalizom (HD) su dobro poznati faktori rizika za visok kardiovaskularni morbiditet i mortalitet ove populacije. **Metode.** Sprovedena je unicentrična, randomizirana prospektivna studija da bi se ispitala moguca korist primene aktivne kon-

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trole volemije (AKV), a na osnovu njenog merenja bioimpedantnom spektroskopijom (BIS), na srčanu morfologiju i funkciju i na miokardnu mehaniku kod hroničnih HD bolesnika. U studiji je učestvovalo 83 HD bolesnika kojima nikada ranije nije rađeno merenje volemije BIS-om i studija je trajala devet meseci. Srčana struktura i funkcionalne karakteristike procenjivane su dvodimenzionalnom Dopler ehokardiografijom, a globalno naprezanje *speckle-tracking* modalitetom. Određivani su nivoi kardioloških markera u krvi. **Rezultati.** Studiju je završilo 73 bolesnika (38 u aktivnoj – AKV grupi i 35 u kontrolnoj grupi). Na kraju studije, glavna strukturna promena u aktivnoj grupi bolesnika bila je redukcija indeksa mase leve komore (62,81 ± 19,74 g/m^{2,7} na početku studije i 57,74 ± 16,87 g/m^{2,7} na kraju studije, $p = 0,007$), dok su glavna funkcionalna poboljšanja u ovoj grupi bolesnika bila poboljšanje ejekcione frakcije leve komore (LVEF, sa 41,27 ± 9,26% na 43,95 ± 8,84%, $p = 0,006$) i njenog frakcionog skraćenja (FS; 27,86 ± 5,94% do 29,86 ± 5,83%, $p = 0,056$), u skladu sa poboljšanjem radijalne mehanike miokarda leve komore registrovanog višim globalnim radijalnim naprezanjem (*strain*-om) (GRS) na kraju studije (18,56 ± 10,24% do 21,79 ± 12,16%, $p = 0,014$). Bolesnici u kontrolnoj grupi imali su značajno pogoršanje dijastolne funkcije procenjeno na osnovu porasta odnosa Doplera brzine ranog dijastolnog punjenja leve komore – E i srednje brzine tkivnog Doplera lateralnog dela mitralnog anulusa – e' (E/e' lateralno; 10,59 ± 5,00 do 11,12 ± 4,06; $p = 0,036$) i posledično, povišenim sistolnim pritiskom u desnoj komori (SPDK, od 34,84 ± 10,18 mmHg do 38,76 ± 8,34 mmHg; $p = 0,028$). Ove funkcionalne promene kod bolesnika u kontrolnoj grupi korelirale su sa značajnim pogoršanjem nivoa N-terminalnog prohormona moždanog natriuretskog peptida (NT-proBNP): medijana i interkvartalni rasopon (IQR) od 5810,0 pg/mL (3339,0–15627,0 pg/mL) na početku studije do 8024,0 pg/mL (4433,0–17467,0 pg/mL; $p = 0,038$), na kraju studije. Poboljšanje srčane morfologije i funkcije u aktivnoj grupi korelirao je sa značajnim smanjenjem procenta „kritično" hipervolemičnih bolesnika na kraju studije (sa 45% na 24%; $p = 0,003$). Na kraju studije, postdijalizno „kritično" dehidriranih bolesnika u kontrolnoj grupi bilo je 49%. Procenat anuričnih bolesnika porastao je samo u ovoj grupi, sa 63% na 77% ($p = 0,063$). **Zaključak.** Koncept aktivne kontrole volemije vođene bioimpedantnom spektroskopijom pozitivno je uticao na hemodinamiku i mehaniku srca kod bolesnika na hroničnom lečenju hemodijalizom i može da pomogne u kliničkom određivanju njihove optimalne težine i daljem kliničkom toku. Potrebni su što pre dodatni podaci o ovom problemu iz dobro dizajniranih studija.

Ključne reči:
dijaliza; funkcija leve komore; ehokardiografija, dopler; bioelektrična impedanca; biomarkeri.

Introduction

The left ventricular myocardial hypertrophy (LVH) and diastolic dysfunction (DD) are dominant cardiac disorders seen in dialysis patients with prevalence 60% to 80% [1–5]. Both disorders are the consequence of hemodynamic (increased preload and afterload) and non-hemodynamic mechanisms (oxidative stress, inflammation, mineral metabolism disturbance etc.) [6, 7]. However, there is still a paradigm that hypervolemia or overhydration (OH) is the main contributing factor for higher blood pressure, LVH and DD among chronic dialysis patients. The main causes of hypervolemia in hemodialysis (HD) patients are oligoanuria, patients' non-compliance and the intermittent nature of HD procedure. There is general consensus that better control of dry weight (DW) in HD patients leads to improved control of hypertension and to left ventricular (LV) mass regression/LV volumes reduction [8–11].

Despite a plethora of methods that have been applied such as measuring inferior vena cava diameter, determination of natriuretic peptides blood level, blood volume monitoring, there is still no ideal and practical method for determining DW [12, 13] and it relies often on conventional clinical assessment [14–16]. Nevertheless, the clinical assessment, alhough rapid and easily applies at the bedside, has its disadvantages [17–20]. There is a growing evidence that bioimpedance spectroscopy (BIS) gives reliable information about OH in dialysis patients [21–27] and correlates well with left ventricular mass (LVM) and cardiomarkers [28]. According to some reports, better control of extracellular water (ECW) by BIS could lead to improved control of arterial hypertension in HD patients [19, 29, 30] and even better management of left ventricular mass in comparison to the standard clinical approach [28, 31]. Still, there are no prospective randomized studies about influence of BIS guided volume control on diastolic function, myocardium mechanical and contractile features in addition to heart morphology and cardiac biomarkers.

The purpose of this prospective randomized study was to investigate possible benefits of the active fluid management (AFM) guided by BIS on cardiac morphology, mechanics and function in chronic hemodialysis patients.

Methods

Study population

This single center, prospective, randomized study included 136 patients on regular HD in the Dialysis Unit of Zvezdara University Medical Center in Belgrade, during the period from February 2013 to August 2014. The study protocol was approved by the Ethical Committees of the Faculty of Medicine, University of Belgrade and the Zvezdara University Medical Center. All participants gave written informed consent to participate in the study. A study schema is presented in Figure 1. From the patients screened, 83 patients fulfilled inclusion/exclusion criteria and were enrolled in the study. The randomization 1 : 1 was made by using online program available at URL http:/www.graphpad.com/quickcalcs/randomize1.cfm and patients were randomized either to the active (n = 42) or to the control group (n = 41). Nine months after enrollment in the study, 38 patients from the active and 35 patients from the control group completed the study.

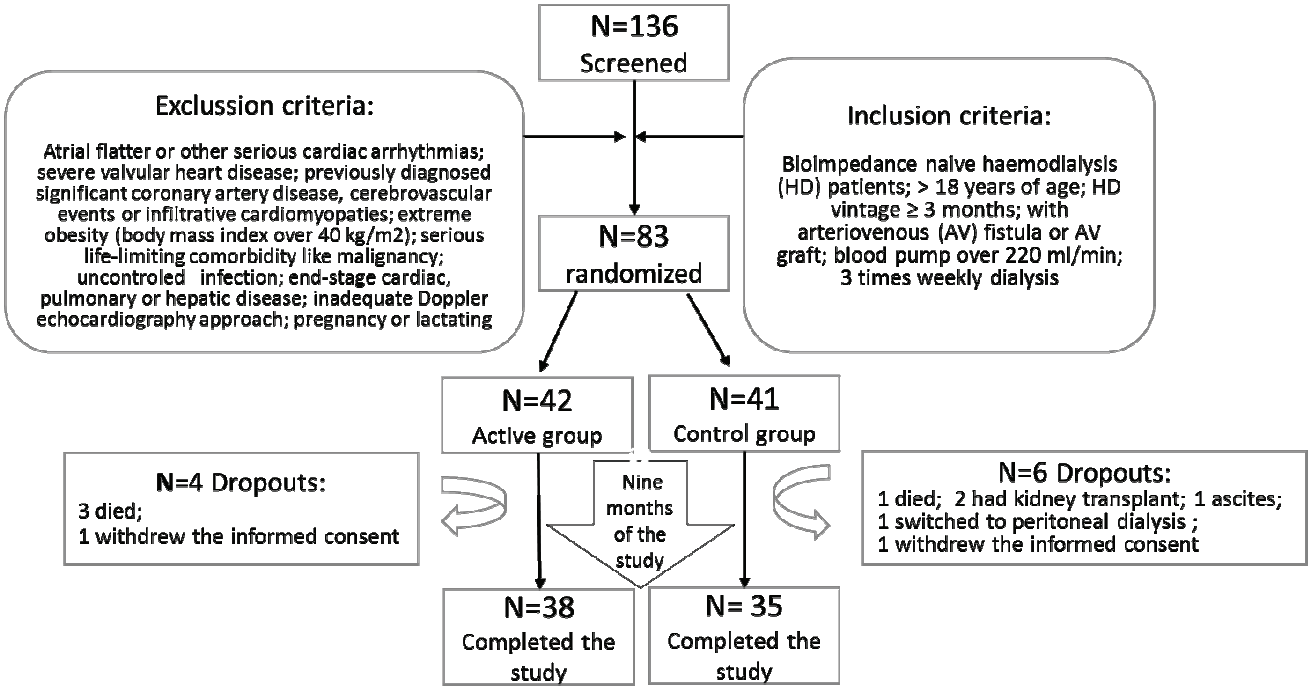

Fig. 1 – Exclusion and inclusion criteria and the study scheme.

Determination of hydration and concept of active fluid management

Hydration status was determined by BIS method implemented in the Body Composition Monitor (BCM, Fresenius Medical Care, Germany). The principles of the technique, validation and clinical implementation have been described elsewhere [18–22, 32]. Volemia is determined by using a physiologic model as a model of normal tissue hydration [22]. The BCM gives OH in liters and suggestion of normal weight (NW) for any particular patient. As well as the determination of OH, the BCM provides information about adipose tissue mass (ATM) and lean tissue mass (LTM). The BCM is routinely used for assessing body composition in many dialysis centers [26, 27].

To overcome the problem of measuring hydration on different sessions of the week which generally results in different OH levels, the concept of average weekly OH (AWOH) was introduced. The basic assumption for application of AWOH is that ATM and LTM remain constant over the period of a week. In the case of thrice weekly HD, only one BCM measurement on any session day of the week is needed. The remaining two OH values are calculated from pre-dialysis weights (preHD_W) and NW:

$OH_{D-1} = preHD\_W_{D-1} - NW$;

$OH_{D-2} = preHD\_W_{D-2} - NW$;

where D-1 and D-2 stands for dialysis sessions prior to dialysis (D) when BCM measurement was conducted.

AWOH is then equal to $(OH_{D-2} + OH_{D-1} + OH_D)/3$

The AWOH was then normalized to ECW to cater for subjects of differing weight and body composition.

Average weekly relative OH (Av_ROH):AWOH/ECW is given in percentage.

Post-dialysis over- or underhydration (postOH) was calculated from post-dialysis weight measurements:

postOH = NW-Weight after dialysis session

Average relative postOH (Av_postROH) was then:

$(OH_{D-2} + OH_{D-1} + OH_D)/3$*ECW (in percentage).

An AFM process was devised for application in those patients enrolled in the active group. This process aims to maintain the pre-dialysis Av-ROH in active patients below 15% as this threshold was considered critical for increased risk of cardiovascular morbidity and mortality in the HD population [18, 33]. A post Av-ROH of -6% was applied to limit dehydration based on previous studies in order to avoid patients' simptoms of dehydration, lower quality of life and to preserve their residual renal function [18, 31, 34, 35], although firmer evidence for this threshold is lacking.

The AFM concept summary and algorithm are provided in Figure 2.

The implementation of active fluid management and body composition monitor

After BCM measurement, in the active group, DW was targeted according to the AFM algorithm and clinical judgment. In the control group, DW was determined only according to routine clinical assessment. The BCM measurements were also performed in this group, but the results remained blinded to the responsible physician.

The BCM measurement was undertaken prior to the start of dialysis treatment, by trained nurses. In the active study group, BCM was performed weekly or monthly, based on the flowchart in Figure 2. In the control group, BCM was performed monthly. Blood pressure (BP) was taken manually before the connection on HD and after the HD session, in the recumbent position.

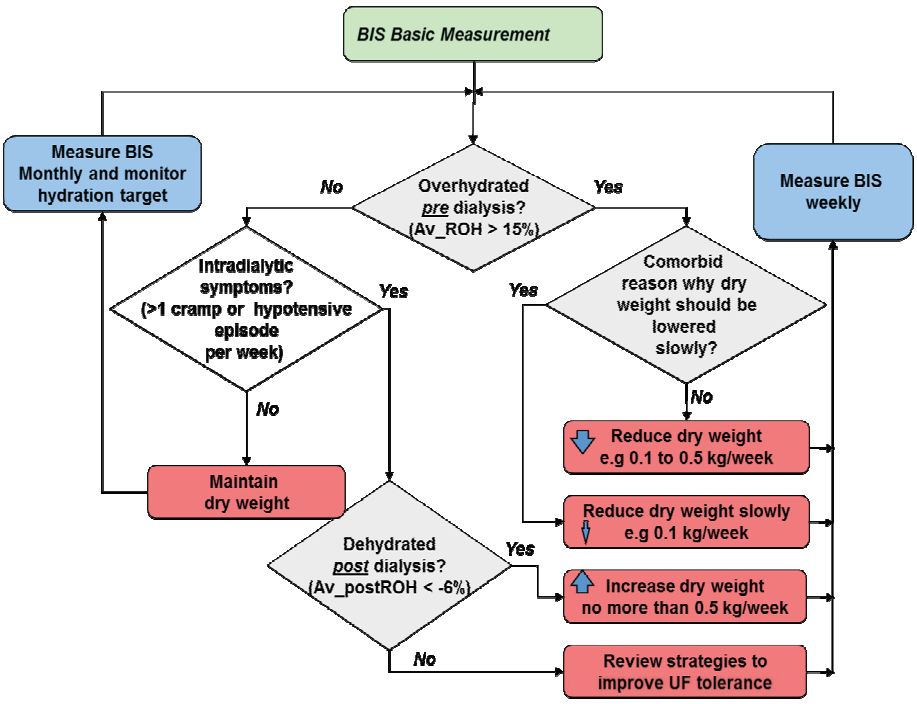

Fig. 2 – Active fluid management flowchart.
BIS – bioimpedance spectroscopy; Av_ROH – average weekly relative overhydration (OH); Av_postROH – average relative post-dialysis over- or underhydration; UF – ultrafiltration.

The mean value of BP measurements at time of BCM and five treatments before BCM were calculated for subsequent analysis. Weight gain was measured as the difference between pre-dialysis weight and clinically targeted patient's DW. An average of three weekly weight gains were divided by the DW as a relative average weight gain (WG_Av) and recorded as a percentage value.

Echocardiography

The echocardiographic examinations at the start and at the end of the study (i.e. 9 months after enrollment) were performed in all patients one day after dialysis in order to avoid the impact of ultrafiltration or pre-dialysis fluid on these measurements, as recommended [36].

The examinations were performed by a cardiologist who had no knowledge to which group (active of control) patients were enrolled. The assessment was done using Toshiba ARTIDA Aplio Ultrasound Machine using 2–4.2 MHz phase array probe for cardiac study in accordance with the recommendations of the European and American Society of Echocardiography [37].

Using M mode images, LV end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD) were obtained as well as intraventricular septum thickness (IVST) and posterior wall thickness (PWT). Relative wall thickness (RTW) was calculated by the standard formula RWT = 2 PWT/LVEDD. Left ventricular volumes (LVEDV and LVESV) were measured using the modified Simpson's method from the apical 4 - and 2-chamber views and indexed by body surface area (BSA) (LVEDVI and LVESVI) and ejection fraction was calculated (LVEF). The similar method, the apical 4 - and 2-chamber endocardial tracing was used for the calculation of the left atrial volume (LAV) and LAV indexed by BSA (LAVI) [37]. LVEF was considered normal if it was ≥ 50% [38].

Left ventricular mass (LVM) was calculated using the Devereux formula[39] and indexed by BSA (LVMI) and by height (h) raised to an exponential power of 2.7 ($LVMI^{2.7} = LVM/h^{2.7}$) [2, 40]. The LVH was defined on the basis of $LVMI^{2.7}$ greater than 48 g/m $^{2.7}$ for men and 44g/m$^{2.7}$ for women [17]. The changes in LVM were also observed. A change greater than 5% of $LVMI^{2.7}$ at the end of the study was considered clinically significant.

Parameters of LV diastolic functions were: peak early (Vmax E), late (Vmax A), and annularlateral and medial e' wave velocity (e' lat and e' med respectively) as well as E/A ratio and E/e' ratio. The parameter E/e' ratio was interpreted as an indirect measure of left ventricular end-diastolic pressure.

The following criteria were applied for the identification of diastolic dysfunction (adapted from Pecois-Filho [7]: E/A ratio < 0.8 or > 2; e' ≥ 8 cm/s; average E/e' ≥ 8; LAVindex > 34 mL/m^2). For the assessment of the structural and functional performance of the right ventricle (RV), the following measurements were used: RV dimension at 4 chamber view, tricuspid annular plane systolic excursion (TAPSE), right ventricular systolic pressure (RVSP), and tricuspid regurgitation [37].

The additional measurement of inferior vena cava (IVC) antero-posterior diameter was performed at the end of the echocardiographic examination in supine position from sub-costal approach within 2.5 cm of IVC - right atrium junction, during unforced breathing. From the recorded frames loops, passive maximal (IVCmax) and minimal IVC

diameter (IVCmin) were obtained (i.e. without sniffing, in order to avoid differences in the magnitude of the inspiratory effort which can influence IVC collapsibility) [41, 42].

The indexed IVCi was calculated by dividing the IVCmax (in mm) by the BSA (in m^2). The IVC collapsibility index (IVC-CI; in %) was calculated by using the following standard formula: [(IVCmax − IVCmin)/IVCmax] × 100.

*Two dimensional (2D) myocardial deformation by speckle tracking***:** Myocardial tissue deformation (strain) was calculated during systole by speckle tracking echocardiography using Toshiba 2D Tissue Tracking system. All global deformation indices were calculated from cardiac cycles acquired and digitally stored on hard disc using an off-line analysis. Global longitudinal strains (GLS) as a reflection of longitudinal endocardial LV mechanics were measured from three conventional apical imaging planes at the end of systole; peak systolic strain was defined as the highest deformation at each plane and the average value was calculated [43]. Global radial strain (GRS) as an index of radial myocardial shortening was obtained from short axis view at the papillary muscle level [44].

Cardiac biomarkers

Plasma samples for determining N-terminal prohormone brain natriuretic peptide (NT-pro-BNP), high-sensitivity C-reactive protein (hs-CRP) and troponin T (TnT) were taken at the start and at the end of the study period. The samples were taken before the dialysis session, from the arterial blood line, one day after echocardiographic assessment. At the same day, the samples were analyzed in the local reference laboratory.

Troponin T was determined by a "sandwich" electrochemiluminescence immunoassay (ECLIA) method, on automatic analyzer (Cobas 501; Roche Diagnostics, Mannheim, Germany). The hs-CRP was measured using an immunoturbidimetric method (Cobas c501; Roche Diagnostics, Mannheim, Germany). The NT-proBNP was measured by a "sandwich" ECLIA method on the Cobas e 411 analyzer (Roche Diagnostic, Mannheim, Germany).

Biochemical parameters

Biochemical parameters were analyzed in the local laboratory from blood collected during the routine patient round before the second weekly session, at the start and at the end of the study including: hemoglobin, albumin, total iron-binding capacity (TIBC), urea, creatinine, intact parathyroid hormone (iPTH), calcium (Ca), phosphate (P), cholesterol (C), low-density lipoprotein-C (LDL-C), high-density lipoprotein-C (HDL-C) and triglycerides. A dialysis dose – the product of the urea clearance (K) over the urea distribution volume (V), and the dialysis session length (t), i.e. Kt/V was measured as single pool Kt/V according to the Daugirdas' formula [45]. During the study, all subjects continued to take their regular medications as indicated by referring doctors. Dialysis modality and dialysis prescription remained constant during the study unless referring doctor changed it based on his/her clinical judgment.

Data collection and statistical analysis

All patients were assigned identification codes to maintain confidentiality. Blood pressure data, HD prescription and all HD treatment data were abstracted from Dialysis charts and entered into an online database created for this study.

Statistical analysis was conducted using IBM SPSS Statistics 19.0 computer program (IBM, USA, 2011). All continuous variables were described in the form of the mean ± standard deviation (SD) except biomarkers which were given as median [interquartile range (IQR)] values. The categorical variables were expressed as percentages and examined using the χ^2 test; the Yates's correction for continuity was used for 2×2 contingency table. Relationship between variables was tested by Pearson's coefficient correlation. Intragroup comparisions of parametric continuous variables were performed by the paired *t*-test; for non-parametric variables the Wilcoxon signed rank test was used. In addition, the McNemar test was used to compare results of binary variables at baseline and at the end of the study. Comparisions of parametric variables between 2 groups were performed by independent *t*-test; non-parametric variables were tested with the Mann-Whitney *U* test. The normality distribution of data was tested by the Shapiro-Wilk test (subject number in the group less than 50). All the analyses were evaluated at the level of statistical significance of $p < 0.05$.

Results

Baseline characteristics

Patients in both groups were of similar age, predominantly male with high prevalence of hypertension (Table 1). The LVH assessed by basic echocardiography had 69 out of 83 patients (83.1%). Left ventricular hypertrophy and hypertension were not correlated with presence of residual renal function ($r = -0.043$; $p = 0.702$ and $r = 0.120$; $p = 0.279$, respectively).

Diastolic dysfunction was registered in 77 (92.1%) patients (Table 1). Arterial pressure in both groups was similar. Also, Av_ROH, Av_postROH and WG_Av were similar between the groups. The "critical" post-dialysis dehydration (Av_ROH < -6%) was correlated with Wg_Av > 5% ($r = 0.223$; $p = 0.049$) in the whole study group.

Of the 83 that enrolled, 73 patients completed the study, including 38 patients (24 males) in the active group and 35 patients (21 males) in the control group (Figure 1).

Patients in the active group exhibited a significant reduction in volume overload from baseline to the end of the study: 45% of active patients were found to have an Av_ROH > 15% at baseline while at the end of the study only 24% were above the 15% Av_ROH threshold.

Table 1

Baseline patients' characteristics

Parameters	Active group (n = 42)	Control group (n = 41)	*p* value
Age (years), mean ± SD	56.1 ± 11.5	57.5 ± 13.2	0.596
HD vintage (months), mean ± SD	79.9 ± 59.2	95.3 ± 80.0	0.600
Males, %	59.5	56.1	0.925
Arterial hypertension, %	76.2	70.7	0.573
Diabetes mellitus, %	11.9	7.3	0.737
Smokers, %	59.5	43.9	0.190
Diuresis ≥ 200 mL/24 h, %	28.6	34.1	0.756
HD session duration (hours, weekly), mean ± SD	12.5 ± 1.0	12.4 ± 1.1	0.814
Blood pump rate (mL/min), mean ± SD	277.2 ± 22.2	267.0 ± 25.6	0.422
Dialysate sodium (mmol/L), mean ± SD	142.1 ± 2.5	142.9 ± 2.7	0.178
HDF, %	33.3	19.5	0.214
Av_ROH (%), mean ± SD	11.8 ± 8.0	12.4 ± 7.0	0.702
Av_ROH > 15%, %	45.2	31.7	0.261
Av_postROH < - 6%, %	42.9	43.9	1.000
WG_Av (%), mean ± SD	4.5 ± 1.4	4.6 ± 1.7	0.751
LVH, %	81	85.4	0.770
DD, %	90.5	91.5	0.676
MAP pre HD (mmHg), mean ± SD	92.5 ± 10.0	88.4 ± 11.3	0.083
MAP post HD (mmHg), mean ± SD	84.6 ± 11.8	81.1 ± 10.7	0.158

HD – hemodialysis; HDF – hemodiafiltration; DD – diastolic dysfunction; Av_ROH – average weekly overhydration; Av_postROH – average weekly post dialysis overhydration; LVH – left ventricular myocardial hypertrophy; DD – diastolic dysfunction; AP pre HD – pre-hemodialysis mean arterial pressure; MAP post HD – post-hemodialysis mean arterial pressure; SD – standard deviation.

Table 2

Dialysis and hydration data in the study patients at enrollment time (0m) and after 9 months of study

Parameters	Active group (n = 38)			Control group (n = 35)		
	0 months	9 months	*p*-value	0 months	9 months	*p*-value
Av_ROH (%), mean ± SD	11.8 ± 8.3	10.3 ± 5.8	0.079	12.2 ± 7.2	11.3 ± 7.2	0.501
Av_ROH > 15%, n (%)	17 (44.7)	9 (23.7)	0.003	11 (31.4)	9 (25.7)	0.774
Av_postROH < - 6 %, n (%)	16 (42.1)	11 (28.9)	0.267	16 (45.7)	17 (48.6)	1.000
MAP pre HD (mmHg), mean ± SD	92.7 ± 10.4	91.2 ± 8.9	0.364	89.7 ± 10.7	91.3 ± 9.2	0.225
MAP post HD (mmHg), mean ± SD	84.6 ± 12.1	85.4 ± 18.0	0.655	81.9 ± 10.2	84.1 ± 12.0	0.235
HDF, n (%)	13 (34.2)	14 (36.8)	1.000	5 (14.3)	7 (20)	0.50
HD duration (hours, weekly), mean ± SD	12.5 ± 1.09	12.53 ± 1.14	0.922	12.43 ± 1.07	12.30 ± 1.02	0.413
Blood pump rate (mL/min), mean ± SD	272.6 ± 21.3	268.0 ± 27.5	0.217	268.1 ± 24.0	267.3 ± 20.6	0.806
Dialysate sodium (mmol/L), mean ± SD	142.1 ± 2.46	140.1 ± 1.7	< 0.001	142.9 ± 2.7	141.3 ± 2.7	0.001
Diuresis (≥ 200 mL), n (%)	10 (26.3)	10 (26.3)	1.000	13 (37.1%)	8 (22.9)	0.063

HD – hemodialysis; HDF – hemodiafiltration; MAP pre HD – pre-hemodialysis mean arterial pressure; MAP post HD – post-hemodialysis mean arterial pressure; Av_ROH – average weekly overhydration; Av_postROH – average weekly post dialysis overhydration.

The number of dehydrated patients increased in the control group, while it was slightly reduced in the cctive group. The residual renal function (RRF) declined only in the control group: 38% patients in this group with RRF function at the start of the study became anuric through the end of the study while there was no new anuric patients in the active group during the study. There was a rise in the pre-dialysis MAP from the beginning to the end of the study in the control group (1.63 mmHg higher after 9 months) while a decrease in MAP was observed in the active group (1.45 mmHg lower after 9 months), however the difference was not statistically significant in either group (Table 2). In both study arms, the dialysate sodium concentration was reduced at the end of the study and it was statistically significant ($p = 0.001$).

Biomarker and biochemistry data

At the end of the study, cardiac biomarkers did not change significantly either in the active or in the control group except for NT-proBNP concentration that significantly increased in control group ($p = 0.038$), (Table 3).

The biochemical parameters of the two groups of patients are shown in Table 4. In the active group a significant decrease was observed for serum albumin level and for TIBC. In the control group, patients had significantly improved hemoglobin, while TIBC, total C, HDL-C and serum P levels all worsened significantly (Table 4).

Table 3

Cardiac biomarkers in the study patients at enrollment (0 month) and after 9 months of study

Parameters	Active group (n = 38)			Control group (n = 35)		
	0 month	9 months	*p*-value	0 month	9 months	*p*-value
hs-CRP (mg/L)	4.02 (1.99–8.55)	4.42 (2.38–9.04)	0.577	3.86 (1.95–6.44)	4.09 (2.38–7.38)	0.169
TnT (μg/L)	0.048 (0.031–0.074)	0.048 (0.031–0.071)	0.689	0.052 (0.038–0.081)	0.052 (0.035–0.077)	0.224
NT-proBNP (pg/mL)	4527.0 (1449.3–10821.8)	4692.0 (1895.8–10033.8)	0.755	5810.0 (3339.0–15627.0)	8024.0 (4433.0–17467.0)	0.038

Biomarkers are given as median (interquartile range – IQR) concentrations.
hs-CRP – high sensitivity C-reactive protein; TnT – troponin T; NT-proBNP – N-terminal prohormone of brain natriuretic peptide.

Table 4

Main biochemical and nutritional parameters in the study patients at the time of enrollment (0 months) and at the end of the study period (9 months)

Parameters	Active group (n=38)			Control group (n=35)		
	0 months	9 months	*p*-value	0 months	9 months	*p*-value
Hb (g/dL), mean ± SD	10.5 ± 1.5	10.7 ± 1.4	0.609	9.9 ± 1.7	10.6 ± 1.7	0.032
Albumin (g/L), mean ± SD	40.3 ± 2.9	38.1 ± 4.0	< 0.001	39.2 ± 3.4	38.2 ± 2.9	0.092
TIBC (μmol/L), mean ± SD	41.5 ± 6.9	38.7 ± 7.1	0.007	40.3 ± 5.9	37.6 ± 8.8	0.012
Cholesterol (C) (mmol/L), mean ± SD	4.93 ± 1.10	5.07 ± 1.07	0.323	4.75 ± 0.73	4.32 ± 0.75	0.001
HDL-C (mmol/L), mean ± SD	1.08 ± 0.43	1.12 ± 0.50	0.509	1.07 ± 0.34	0.90 ± 0.28	< 0.001
spKt/V, mean ± SD	1.50 ± 0.32	1.54 ± 0.33	0.456	1.40 ± 0.26	1.40 ± 0.19	0.932
Ca (mmol/L), mean ± SD	2.31 ± 0.27	2.26 ± 0.24	0.083	2.34 ± 0.20	2.36 ± 0.22	0.837
P (mmol/L), mean ± SD	1.56 ± 0.52	1.47 ± 0.44	0.489	1.79 ± 0.54	1.60 ± 0.48	0.005
iPTH (pg/mL), median (range)	259.9 (100.8–588.1)	197.4 (97.5–482.9)	0.067	293.6 (106.0–582.8)	192.2 (78.0–625.2)	0.404

Hb – hemoglobin; TIBC – total iron binding capacity; HDL – high density lipoprotein; spKt/V – single pool Kt/V; P – phosphate; Ca – calcium; iPTH – intact parathyroid hormone.

Table 5

Doppler echocardiographic indices of cardiac structure and function in study arms at the enrollment (0 m) and after 9 months (9 m)

Parameters	Active group (n = 38)			Control group (n = 35)		
	0 months	9 months	*p*-value	0 months	9 months	*p*-value
LAV index (mL/m^2)	31.24 ± 11.61	29.57 ± 11.60	0.301	33.17 ± 12.39	35.37 ± 12.15	0.229
LVEDD (cm)	57.29 ± 6.03	55.97 ± 7.19	0.222	54.39 ± 6.31	55.72 ± 6.06	0.136
LVESD (cm)	41.39 ± 6.73	39.07 ± 6.85	0.024	37.23 ± 7.35	37.72 ± 6.73	0.669
LVEDV index (mL/m^2)	71.73 ± 23.83	65.55 ± 22.18	0.103	71.73 ± 23.82	65.55 ± 22.18	0.576
LVESV index (mL/m^2)	40.19 ± 12.50	35.79 ± 13.53	0.023	34.59 ± 11.53	36.7 ± 14.82	0.665
LVEF (%)	41.27 ± 9.26	43.95 ± 8.84	0.006	45.66 ± 8.74	44.32 ± 9.34	0.292
LVEF ≥ 50 (%)	8 (21.1%)	12 (31.6%)	0.125	11 (31.4%)	8 (22.9%)	0.508
RWT	0.384 ± 0.070	0.387 ± 0.071	0.800	0.401 ± 0.065	0.394 ± 0.047	0.476
LVM index (g/m^2)	147.09 ± 42.12	133.80 ± 33.57	0.003	135.68 ± 29.39	139.93 ± 35.72	0.372
LVMI index$^{2.7}$ (g/m$^{2.7}$)	62.81 ± 19.74	57.74 ± 16.87	0.007	60.35 ± 13.06	62.55 ± 16.97	0.301
FS (%)	27.86 ± 5.94	29.86 ± 5.83	0.056	30.71 ± 7.06	31.63 ± 5.52	0.466
E/e' med	12.52 ± 6.79	11.99 ± 3.75	0.690	12.68 ± 4.54	13.21 ± 4.10	0.342
E/e' lat	10.35 ± 4.73	9.96 ± 3.43	0.777	10.59 ± 5.00	11.12 ± 4.06	0.036
GLS LV Strain (%)	-9.56 ± 3.96	-10.37 ± 4.02	0.118	-10.18 ± 3.97	-10.28 ± 4.26	0.888
RS LV Strain (%)	18.56 ± 10.24	21.79 ± 12.16	0.014	24.21 ± 13.62	22.43 ± 12.07	0.550
Right Ventricle (mm)	35.10 ± 7.56	35.13 ± 7.35	0.983	35.38 ± 6.40	36.06 ± 7.37	0.598
TAPSE (%)	21.66 ± 5.29	21.61 ± 4.06	0.839	22.79 ± 5.72	21.02 ± 3.75	0.136
RVSP (mmHg)	35.69 ± 11.24	35.01 ± 9.30	0.565	34.84 ± 10.18	38.76 ± 8.34	0.028
IVCi (mm/m^2)	7.33 ± 2.58	7.36 ± 2.07	0.949	8.35 ± 2.79	8.97 ± 2.87	0.178
IVC-CI (%)	55.66 ± 24.56	56.20 ± 16.72	0.908	49.35 ± 15.33	49.07 ± 16.01	0.928

Results are given as mean ± standard deviation or number (%) of patients.
LAV index – left atrial volume index; LVEDD – left ventricle end-diastolic diameter; LVESD – left ventricle end-systolic diameter; LVED-VI – left ventricle end-diastolic volume index; LVESVI – left ventricle end-diastolic volume index; LVEF – left ventricle ejection fraction; RWT – relative wall thickness; LVMI – left ventricular mass index; LVMI$^{2.7}$- left ventricular mass indexed by height$^{2.7}$; FS- fractional shortening of the LV; GLS - Global longitudinal strain; GRS - global radial strain; TAPSE- tricuspid annular plane systolic excursion; IVC- inferior vena cava; IVCi- inferior vena cava index; IVC-CI- inferior vena cava collapsibility index; E/e' med - ratio of the peak trans-mitral filling velocity early in diastole (E wave) and the early relaxation LV velocity measured on medial (septal) part of the mitral annulus (e'med); E/e' lat- E/e' ratio where e' is measured on lateral part of the mitral annulus; RVSP - right ventricle systolic pressure

Hemodynamic data

The average LVEF was improved after 9 months in the active group [from 41.27 to 43.95%, ($p = 0.006$)] and this difference was not observed in the control group (Table 5). In addition, patients from the active group significantly improved their LVESD ($p = 0,024$), LVESVI ($p = 0.023$), LVMI ($p = 0.003$), $LVMI^{2.7}$ ($p = 0.007$) and GRS LV strain ($p = 0.014$). In the control group, patients significantly increased E/e' lat and RVSP, indicating worsening of diastole LV function. Other parameters remained unchanged (Table 5).

The main structural changes in the active group of patients were a reduction of LVMI as well as $LVMI^{2.7}$, while main functional improvements after 9 months of AFM was better LVEF and FS in accordance to improvement of radial LV mechanics detected by higher GRS.

Discussion

This study confirmed that our HD patients had very high prevalence of LVH and diastolic dysfunction (83% and 92%, respectively) along with high average weekly OH. During the study, patients in the active group significantly improved their overhydration but also several cardiac parameters including MAP, LVEF, LVESD, LVESV index, LVM index, LVM $index^{2.7}$, and GRS LV strain. On the other hand, patients in the control group, managed by routine clinical assessment, exhibited a deterioration of diastolic function and, consecutively, RVSP. These functional changes were associated with significantly higher levels of NT-proBNP in this group of patients. These findings were associated with better Av_ROH management in the active group, and the percent of "critically" overhydrated patients decreased from 45% to 24% from baseline to the end of the study period. The reduction of critically overhydrated patients by BIS guided fluid management is consistent with the findings of others [19, 29, 46].

There are numerous studies concerning the degree of hydration and cardiovascular impairment in end stage kidney disease (ESKD) patients [8, 9, 27, 29–31], as well as about the association of chronic fluid overload assessed by BIS with LVM and level of cardiac biomarkers [28]. However, to the best of our knowledge, there is no prospective study addressing the influence of DW probing either by BIS or by other methods to LV performance, especially LVM with myocardial mechanics and cardiomarkers. There are few prospective randomized studies that used BIS measurements to target post-dialysis weight which showed significant improvement in LVM and/or blood pressure regulation although the BIS measurements were performed twice monthly [30] or even less frequently [31]. A study by Moissl et al. [29] addressed the issue of using one standard protocol for the implementation of BIS measurements in clinical practice for patients on chronic HD program. That study was not randomized, lasted 3 months, and echocardiography was not performed.

Patients from whole study group had global myocardial strains values below the normal range. Lower contractility was reflected by lower LVEF at the beginning of the study (74% of patients had LVEF under 50%). Therefore, it is not surprising that strains, as indicators of LV contractility [47, 48] are far below the normal. The normal level of GLS in the general population is < -18% [44], but Krishnasamy et al. [38] suggested a level of GLS < -16% as normal for HD patients population. If we had used this criteria for GLS, there would have been just 6 of 73 patients at the start and only 3 at the end of the study with normal values in both groups. Although GLS, in general, did not improve much during the study in the active group, radial contractility, expressed by GRS, significantly enhanced, suggesting that optimal changes in volume load influenced primarily radial myocardial shortening with pump function upgrading.

Patients in the control group had deteriorated diastolic function as assessed by E/e' raise and also of RVSP, and this was followed by an increase of NT-proBNP. Moissl et al. [29] did not observe any improvement in BNP level in their study after 3 months and based only on volemia criteria. However, there was no data regarding LVM, or degree of diastolic dysfunction in observed population. In addition to volemic status, one could speculate that NT-proBNP correlates with diastolic dysfunction as well [49].

It is important to mention that apart from overhydration before HD, there was a high proportion of post-dialysis dehydrated patients (i.e. with Av_postROH< -6%) in both groups (over 43%) at the start of the study. According to our results, almost every second patient in the control group (49% of them) was dehydrated after dialysis more than 6% of their ECW at the end of the study. In other studies, the proportion of such underhydrated patients was smaller – in a range from about 3% to 30% [19, 26, 29, 46, 50]. One possible explanation is that most of the patients in this study had a long dialysis experience and wished to avoid overt overload syndrome, so they refused increases in their dry weight. Also, post-dialysis dehydration closely correlated with higher weight gain (i.e. "overhydration" in terms of clinically targeted DW) indicating a limitation of a thrice weekly dialysis schedule. The consequences of post-dialysis dehydration are not clearly described in the literature but it may influence patients' quality of life and expose them to a risk of hypotension and its consequences. Still, our dehydrated patients in both study groups did not change their MAP significantly which cannot be explained by our study protocol. Therefore, our experience indicates that DW needs to be established with care. This process is time-dependent and requires a full compliance from the patient before achieving the goal.

When establishing the appropriate dry weight for an individual patient, the influence on residual renal function must be taken into account. According to our experience, patients in the active group maintained their residual diuresis during the study period. However, there was a significant decrease in the proportion of the patients with residual diuresis in the control group. Our results demonstrated a significant reduction in dialysate sodium among patients in both study arms. As was mentioned previously, daily visits by physicians include the monitoring of DW and dialysis parameters including dialysate sodium. Therefore, it was not surprise that the control group had fewer extremely overhydrated patients at

the end of the study as compared with the study start. These cofactors may influence the overall results of the study.

Finally, deterioration of some nutritional parameters (serum albumin and TIBC) could be explained by stricter dietary control during the study. The values of serum albumin and TIBC remained in the reference range but did not suggest any malnutrition.

Limitations of the study

It was single center study that included a relatively small sample of the participants. Cardiac structure, function and mechanics was performed only by 2D echocardiography without other imaging techniques.

Conclusion

Bioimpedance spectroscopy measurements implemented through the active fluid management concept had positive impact on cardiac hemodynamics and mechanics in our study patients. Comprehensive evaluation of cardiac structure/function and cardiac biomarkers shed more light in determining dry weight in dialysis patients.

Active fluid management in everyday clinical practice could improve clinical decisions regarding optimal weight and further clinical course in hemodialysis patients. Well designed studies are needed urgently to investigate the value of guided fluid management approaches.

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Disclosures

MDM, NMN, NR and NR declare no conflict of interest with the content of this manuscript. ZP is the employee of the Special Hospital for Hemodialysis "Fresenius Medical Care" Belgrade. "Fresenius Medical Care" is the manufacturer of the BCM device and was not involved in the design or conduct of the study.

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The impact of altered hands function on working ability of patients with systemic sclerosis

Uticaj izmenjenih funkcija šaka na radnu sposobnost obolelih od sistemske skleroze

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Abstract

Background/Aim. Hand impairment in systemic sclerosis (SSc) patients is the most frequent manifestation of this progressive disease and often cause problems in daily activities and working ability. The correlation of altered hand functions in patients with SSc and their impact on working ability is not fully explained. The objective of this study is to assess the impact of altered hand functions of patients with SSc on their working ability. **Methods**. We assessed 20 patients with SSc (17 females, 3 males), with mean age of 45.5 ± 11.9 years (min 29, max 69, Med 44.0 years). The movements of fingers, wrist and forearm were examined by measuring active range of motion of the hand and fingers, muscle strength of the fingers, the skin lesions by modified Rodnan score, the function of the fingers, hands and forearms by the Hand Mobility in Scleroderma (HAMIS) test, as well as the condition of the capillaries in the fingers by capillaroscopy and working capacity by Work Ability Index (WAI). **Results.** Reduced strength on at least one tested muscle, was established in all patients, thickening of the skin on the hands in 14/20 (70%) and the fingers in 19/20 (95%), "scleroderma type" capillary changes in 15/17 (85%), decreased range of motion in finger joints in 16/20 (80%) of the patients. Also, 14/20 (70%) of subjects reported problems at work [decrease performance achieved in 8/20 (40%), reduction of working hours in also 8/20 (40%), leaving out some of the work in 14/20 (70%), and investing of extra effort in 15/20 (75%)]. By means of WAI, work ability assessment questionnaire, it was found that the working capacity was reduced in 11/20 (55%) of our patients. None of the patients was in the group of the most serious cases, the "poor" category; in the "moderate" category there were 6/20 (30%), while 5/20 (25%) of the patients were in the group with minimally reduced working capacity ("good" category). There was a statistically significant correlation between the thickened skin on the fingers ($p < 0.05$), reduced muscle strength in the fingers ($p < 0.002$) and limited mobility of the individual finger joints ($p < 0.05$), with information on reduced working capacity obtained by means of WAI questionnaire and with answers to questions about problems at work. **Conclusion.** The thickened skin on the fingers, reduced fingers muscle strength and limited mobility of certain finger joints in patients with SSc cause decreasing working capacity for all jobs that include manual activities.

Key words:
scleroderma, systemic; work capacity evaluation; hand.

Apstrakt

Uvod/Cilj. Oštećenje šaka kod obolelih od sistemske skleroze (SSc) je najčešća manifestacija ovog progresivnog oboljenja i često uzrokuje probleme tokom svakodnevnih aktivnosti i radnih obaveza. Povezanost izmenjenih funkcija šaka obolelih od sistemske skleroze i njihov uticaj na radnu sposobnost nije u potpunosti objašnjen. Cilj ovog rada je da se kod obolelih od SSc ispita povezanost izmenjenih funkcija šaka sa njihovom radnom sposobnošću. **Metode**. Ispitano je 20 bolesnika sa SSc (17 ženskog i 3 muškog pola), prosečne starosti 45,5 ± 11,9 godina (min 29, max 69, Med 44,0 godine). Ispitana je pokretljivost prstiju, ručja i podlaktice kroz merenje aktivnog obima pokreta šake i prstiju, snaga mišića prstiju ruku, rasprostranjenost kožnih promena modifikovanim Rodnanovim kožnim skorom, funkcije prstiju, šaka i podlaktica *Hand Mobility in Scleroderma* (HAMIS) testom, stanje kapilara na prstima ruku kapilaroskopijom i procena radne sposobnosti primenom *Work Ability Index* (WAI). **Rezultati.** Smanjena mišićna snaga na bar jednom ispitivanom mišiću, utvrđena je kod svih ispitanika, zadebljanje kože na šakama kod 14/20 (70%), na prstima kod 19/20 (95%), promene kapilara "sklerodermnog tipa" kod 15/17 (85%), smanjen obim pokreta u zglobovima prstiju kod 16/20 (80%). Na probleme na poslu žalilo se 14/20 (70%) ispitanika [na smanjenje postignutog učinka (8/20)

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(40%), na skraćenje radnih sati, takođe, 8/20–40%, na izostavljanje nekih radnji 14/20 (70%), na ulaganje dodatnog napora 15/20 (75%)]. Procenom radne sposobnosti WAI upitnikom utvrđeno je da je radna sposobnost umanjena kod 11/20 (55%) ispitanika. U grupi najtežih slučajeva, kategoriji „loše“, tj. ispitanika gde je bila izraženo umanjena radna sposobnost, nije bilo nijednog ispitanika; u grupi koja pripada kategoriji „osrednje“ bilo ih je 6/20 (30%) i u grupi sa najmanje umanjenom radnom sposobnošću (kategorija “dobro”) 5/20 (25%) ispitanika. Nađena je statistički značajna povezanost između zadebljale kože na prstima ruku ($p < 0,05$), smanjene mišićne snage prstiju ($p < 0,002$) i ograničene pokretljivosti pojedinih zglobova prstiju ($p < 0,05$), sa podacima o umanjenoj radnoj sposobnosti dobijenim WAI upitnikom i sa odgovorima na pitanja o problemima na poslu. **Zaključak.** Zadebljala koža na prstima ruku, smanjena mišićna snaga prstiju i ograničena pokretljivost pojedinih zglobova prstiju kod bolesnika sa SSc uzrokuju smanjenje radne sposobnosti za sve poslove vezane za manuelne aktivnosti.

Ključne reči:
sklerodermija, sistemska; sposobnost, radna, ocena; ruka.

Introduction

Systemic sclerosis (SSc) is a chronic, systemic, progressive, autoimmune disease, characterized by lesions on the small blood vessels, excessive deposition of collagen and other extracellular connective tissues in the skin, locomotor system and some internal organs. Etiology is unknown, but genetic predisposition plays an important role in development of fibroproliferative changes [1–4].

The correlation of altered hand functions in patients with SSc and their impact on working capacity is not fully explained. The results of a number of previous studies which were conducted both in our country and worldwide, were not coherent, and controversial conclusions are explained by different methodological approaches and by evaluating insufficiently specific and characteristic parameters, stages of the disease in which the studies were performed, effects of various therapeutic procedures at the time of testing, and the individual characteristics of subjects.

There are many indicators of the functioning of the hand: anatomical integrity, mobility, muscular strength, sensitivity, capturing function, accuracy, coordination, unilateral and bilateral tasks, daily activities. None of the methods of assessment includes all the functions of the hand.

Working capacity implies appropriate anatomical and functional condition of organs and systems that enables to fully comply with all duties related to occupational or daily activities. It also means the ability of an organism to maintain the internal physiological balance during operation, and after the termination of work promptly and fully establish all forms of balance that deviated from the physiological values [5].

The objective of this study was to evaluate the correlation between altered function of the hand in patients with systemic sclerosis with their working capacity.

Methods

The study was conducted at the Institute of Rheumatology in Belgrade, Serbia and included thorough exam of 20 adult patients (17 women and 3 men) with a confirmed diagnosis of SSc without other systemic connective tissue diseases. All subjects were aware of the aim of this study and signed a voluntary consent to participate, and the two independent Ethic Committees approved its implementation.

Evaluation of health status of the patients was marked from 1 to 5 (1 – excellent, 2 – very good, 3 – good, 4 – average, 5 – bad).

Physical pain was marked also from 1 to 5 (1 – no pain, 2 – very weak pain, 3 – weak pain, 4 – moderate pain, 5 – strong pain).

For the evaluation of the extent and severity of skin lesions, the modified Rodnan skin score (mRSS) was used [6]. It is determining skin involvement by palpation of seventeen anatomic sites and scoring on a 0–3 scale (0 – normal finding, 1 – possible skin thickening, 2 – thickened skin not attached to deeper layers, 3 – thickened skin attached to deeper layers). The scores for all sites were summed to give a total skin score, with a minimum possible score of 0, and maximum possible score of 51 points. Of all estimated anatomic sites, only regional skin indicators of four sites (thickening of the skin on fingers and hands, left and right) were taken for further analyses in this study. They were also scored from 0 to 3 (see above). Minimum possible score was 0, maximum was 12 points.

Condition of the capillaries was evaluated semiquantitatively by the Maricq method of capillaroscopy, with minor modifications according to Damjanov [7]. Marks were from 1 to 4 (1 – unspecific changes, 2 – dilated capillaries with minor avascular areas, 3 – dilated capillaries with large avascular areas, 4 – very large avascular areas). For the classification of observed capillary changes, we also used the classification by Cutolo with marks from 1 to 4 (1 – normal findings, 2 – early changes, 3 – active changes, 4 – late changes).

The mobility of the wrist and fingers were monitored by measuring (on both hands) the flexion and extension of the fingers, thumb abduction and adduction, as well as palmar and dorsal flexion (extension) of the wrist. Mobility of the forearm was determined by measuring pronation and supination. Active range of motion (AROM) was measured on both arms in angle degrees at the level of the wrist radiocarpal joint (RC), in the metacarpophalangeal joints (MCP) and interphalangeal finger joints (IP, distal DIP and proximal PIP) and expressed as a percentage of the maximum possible values. In this study, only the mobility of the first three fingers of both hands, which are the most important for the function of capturing, holding and manipulating of small objects, were taken for further consideration.

Testing the strength of individual muscles or muscle groups was performed using manual muscle test (MMT) [8].

From all assessed muscles of both hands, seven of them (important for movements during work or daily activities) were selected and analyzed: *extensor digitorum communis* (EDC), *flexor digitorum superficialis* (FDS), *flexor digitorum profundus* (FDP), *abductores pollicis* (*longus et brevis*) (ABDP), *adductores pollicis* (ADDP), *opponens pollicis et digiti* V (OPP), *flexor pollicis longus* (FPL).

The MMT score was also used as an indicator of changes in the peripheral motor neuron, as an indicator of the degree of hypotrophy/atrophy of muscles and as an indicator of damage to the joints in the vicinity of the test muscle. Muscle strength in our work was marked from 1 to 5 (1 – normal muscle strength, 2 – good, 3 – moderate, 4 – low, 5 – absence). For more accurate statistical comparison, the original MMT marking (5 for normal muscle strength, up to 0 for total loss of muscle strength) was shown in reversed order in this work.

The original Hand Mobility in Scleroderma (HAMIS) test which follows 9 tested items, was applied for evaluation of the remaining functions of the fingers, hands and forearms of both hands (for assessing finger flexion, extension and abduction, thumb abduction, pincer grip – thumb and index finger opposition, wrist extension and flexion, pronation and supination) [9]. In addition to analysis of these values, we separately monitored conditions of the fingers regardless of the wrist, then the fingers with the wrist, as well as the total value of HAMIS test, which included forearm mobility in estimation of both hands. Each item was graded on 0–3 scale where 0 corresponds to normal function and 3 denotes that the individual cannot perform the task. For each hand, total HAMIS score was in range from 0 (normal findings) up to 27 (complete inability to perform tasks representing a high degree of dysfunction).

Assessment of disease severity and functional condition of the hands was also carried out by means of the task-induced fatigue (TIFS) questionnaire Scleroderma Assessment Questionnaire (SAQ) which was developed, validated and published by the doctors from the Institute of Rheumatology in Belgrade as an original tool for assessing the severity of illness and functional capability of patients with SSc as a whole, but only questions directly related to the function disturbance of the hands and fingers were covered in our paper [10]. Those questions were related to ability to hold pencil, to unbutton the shirt, to unscrew the tap, to hold the knife and cut meat, to perform household activities, or to detect pain in fingers while holding objects or exposing to cold weather.

In addition, questionnaire for assessing the quality of life were taken into account in respect of the answers to several questions directly showing impact on the disturbed working capacity (questions detecting if the subjects had to reduce time spent on work and other activities, or to leave out some of the work, or to achieve less than expected, or to invest an extra effort in work or other activities).

Work ability was assessed with the Work Ability Index (WAI) [11–13], which is a self-administered questionnaire comprising seven items considering the demands of work, the worker's health status and resources. The items are grouped according to: (a) estimation of current work ability compared with lifetime best; (b) work ability in relation to physical and mental demands of the work; (c) number of diagnosed diseases; (d) estimation of work impairment due to diseases; (e) sick leave during the past year; (f) own prognosis of work ability after 2 years; (g) psychological resources. For each item, a single-item score is obtained. The final WAI score is calculated as the sum of all single-item scores. Higher scores on the WAI indicate better work ability. Based on this score the individual's work ability can be classified into four categories: 1 – excellent (44–49 points), 2 – good (37–43 points), 3 – moderate (28–36 points) and 4 – poor (less than 28 points).

Statistical analysis

After description of all answers and marks, statistical significance was tested through parametric Student *t*-test, and the nonparametric sum rank test (Mann-Whitney test) for two groups of data. Nonparametric analysis of variance were performed with Kruskal-Wallis test for more than two groups of data. Nonparametric methods were used more often due to quite small sample and the nature of data (scores obtained after point rating of reports, but testing of normal distribution using Kolmogorov-Smirnov test were final judge). In all methods the level of significance was set at 0.05.

Results

The study was conducted on patients with SSc treated at the Institute of Rheumatology in Belgrade. The treatment included 17 (85%) females and 3 (15%) males. Their average age was 45.5 ± 11.9 years (min 29, max 69, Med 44 years) and did not significantly differ in relation to sex. Average disease duration was 6.4 ± 3.7 years (min 1, max 17, Med 4.0): females 6.6 ± 4.0 (min 1, max 17, Med 5.0) years and males 5.0 ± 3.2 (min 1, max 16, Med 4.5) years, and the difference in the disease duration between sexes was not statistically significant ($z = 0.191$; $p > 0.05$). Ten of our patients were employed and also ten of them were unemployed.

The diagnosis of SSc was set according to the recommendations of the European League Against Rheumatism (EULAR), which were revised in 2013 by a joint committee of the American College of Rheumatology (ACR) and EULAR [14].

In assessing their entire health, 19/20 (95%) of our subjects were dissatisfied. The lowest scores for their health gave 9/20 (45%) of the patients.

Moderate to severe pain in the hands and fingers reported up 19/20 (95%) of our subjects (depending on the questionnaire). The pain was provoked by the cold weather, contact with a cold object or emerged spontaneously.

The most common problems were: weakness in the hands and fingers in 11/20 (55%), stiffness in the hands in 14/20 (70%), lack of manual actions – unscrewing taps in 7/20 (35%), dropping objects in 7/20 (35%), inability to hold a pencil in 6/20 (30%) and buttoning buttons in 6/20 (30%) of our patients (Table 1).

Thickening of the skin, as measured by the mRSS was also found in 19/20 (95%) of our subjects on the fingers bilaterally and in 14/20 (70%) on both hands. Thickening of the skin, unattached to the deeper layers (score 2), as well as attached to the

deeper layers (score 3), was found on the fingers in 17/20 (85%), and on the hands in 7/20 (35%) of the patients.

Capillaroscopy demonstrated lesions in the capillaries on fingers of both hands in 15/17 (85%) of our patients („scleroderma type" of capillary lesions, classified as a type II and III according to Maricq). According to Cutolo classification, 3/17 (18%) of the subjects had early changes (early scleroderma sample), 8/17 (47%) active and 3/17 (18%) had late scleroderma.

Reduced hands muscles strength was tested by MMT in all subjects. In relation to the maximum possible maintenance of the strength (100%) the reduction ranged from 10% to 50% and was most frequently registered in the *flexor digitorum profundus* – FDP (R-45%, L-50%), the *flexor digitorum superficialis* – FDS (R-45%, L-50%) and the *flexor pollicis longus* – FPL (R-40%, L-45%) (R-right hand, L-left hand).

Reduced active range of motion in the joints of the fingers of both hands, was registered in 16/20 (80%) of patients. Most frequently it was the case of the bilaterally reduced flexion of the proximal phalanx of the thumb (75%), flexion of the distal phalanx of the thumb bilaterally (70%), extension of the proximal phalanx of the thumb bilaterally (65%) and flexion of the proximal phalanx of the index finger (R-70%, L-65%). The largest reduction in range of motion was accounted for 89% of the maximum, and was registered for the flexion of the distal phalanx of the middle finger and flexion of the distal phalanx of the thumb bilaterally, as well as 88% of flexion of the proximal phalanx of the right index finger.

Function evaluation by means of the HAMIS test showed that 10/20 (50%) of our patients had damaged function of hands, fingers and forearm, ranging from mild (isolated flexion or extension of each finger failure at 6/20–30%) and severe (complete pronation and supination failure of the forearm, and the limited mobility of the majority of fine joints at 4/20–20%) (Table 2).

Table 1

Frequency of subjective problems

Subjective problems	T	P	%	Mean ± SD	Min	Max
Dissatisfaction with health status	20	19	95	3.50 ± 0.94	1	5
Pain in the hands	20	19	95	3.40 ± 1.31	1	5
Pain in the fingers	20	11	55	1.80 ± 0.84	1	4
Weakness in the hands	20	11	55	2.65 ± 1.08	1	4
Stiffness in the hands	20	14	70	2.30 ± 1.08	1	4
Difficulties in manual actions						
unscrewing taps	20	7	35	1.55 ± 0.94	1	4
dropping objects	20	7	35	1.50 ± 0.82	1	4
holding a pencil	20	6	30	1.40 ± 0.68	1	3
buttoning buttons	20	6	30	1.40 ± 0.68	1	3

T – total number of patients; P – number of patients with positive answer; % – percentage of patients with positive answers; SD – standard deviation.

Table 2

Frequency of objective problems

Objective problems	T	P	%	Mean ± SD	Min	Max
Thickening of the skin (mRSS) on						
fingers	20	19	95	2.54 ± 0.76	0	3
hands	20	14	70	1.36 ± 0.96	0	3
Capillary changes on hand fingers						
Cutolo method/score	17	14	82	2.65 ± 0.96	1	4
Maricq method/score	17	15	85	2.53 ± 0.80	1	4
Diminished muscle strenght						
FDP	20	10	50	2.10 ± 1.33	1	5
FDS	20	10	50	2.05 ± 1.31	1	5
FPL	20	9	45	1.85 ± 1.18	1	5
Diminished range of motion						
flexor of thumb distal phalanx	20	14	70	70.85 ± 31.40	11	100
flexor of thumb proximal phalanx	20	15	75	65.65 ± 30.92	14	100
extensor of thumb proximal phalanx	20	13	65	61.05 ± 34.36	14	100
flexor of III finger proximal phalanx	20	14	70	80.00 ± 29.04	11	100
HAMIS test on						
fingers	20	10	50	1.95 ± 0.77	0	11
fingers + hand	20	10	50	3.70 ± 1.28	0	17
fingers + hand + lower arm	20	10	50	4.00 ± 3.74	0	19

T – total number of patients; P – number of patients with positive answer; % – percentage of patients with positive answers; SD – standard deviation; mRSS – modified Rodnan skin score; FDP – *flexor digitorum profundus*; FDS – *flexor digitorum superficialis*; FPL – *flexor pollicis longus*; HAMIS – hand mobility in scleroderma.

Problems at work and difficulties in performing daily activities due to illness, reported 15/20 (75%) of our patients. They tried to solve those problems: by reducing working hours ¯8/20 (40%); by failure to meet standards – 8/20 (40%); by leaving out some of the work – 14/20 (70%); by giving an extra effort at work –15/20 (75%).

The need to cut the time spent at occupational or daily activities had 8/20 (40%) of our subjects. This finding was significantly associated with an impaired function of finger muscles (FPL 2.50 vs. 1.42, z = 2.20, p = 0.041; ABDP 2.25 vs. 1.08, z = 3.74, p = 0.002; ADDP 2.25 vs. 1.17, z = 2.30, p = 0.034), hand muscles (EDC 2.38 vs. 1.16, z = 3.31, p = 0.004; FDS 3.00 vs. 1.42, z = 3.22, p = 0.005; FDP 3.00 vs. 1.50, z = 2.91, p = 0.009), the function of the fingers, hand and forearm checked by means of the HAMIS test (8.25 vs. 1.17, z = 3.34, p = 0.004), as well as more difficult manual actions (unscrewing the tap, 2.00 vs. 1.25, z = 1.85, p = 0.050; fastening buttons, 1.75 vs. 1.16, z = 2.02, p = 0.048).

Also, 8/20 (40%) of our subjects complained to reduction of achieved performance compared to that prior to the disease, which was in statistically significant connection with pain in the hands (3.25 vs 2.25, z = 2.20, p = 0.041), weakness in the hands (2.13 vs. 1.42, z = 2.10, p = 0.050), stiffness of the hands (3.00 vs. 1.83, z = 2.74, p = 0.013), difficult manual activities (fastening buttons 1.75 vs 1.17, z = 2.03, p = 0.050), and thickened skin on the hands (3.50 vs 3.10, z = 2.05, p = 0.050), as well as with the function of the fingers, hands and forearms, which was measured by means of the HAMIS test (3.88 vs. 0.67, z = 2.51, p = 0.022;7.00 vs. 1.50, z = 2.61, p = 0.018;7.50 vs. 1.67, z = 2.51, p = 0.022).

Further, 14/20 (70%) of the patients had to exclude some kind of activities, which was in statistically significant relation with a physical pain in the hands (3.00 vs. 1.83, z = 2.47, p = 0.024) and with reduced range of motion in the joints of the fingers (94.33 vs. 69.57, z = 2.39, p = 0.028).

Also, 15/20 (75%) of our patients had to make additional efforts in implementing working tasks, with statistically significant connection with the subjective assessment of the current state of health (3.80 vs. 2.60, z = 2.89, p = 0.010), physical pain in fingers and hands, (3.87 vs. 2.00, z = 3.45, p = 0.003), as well as with the stiffness of the fingers (2.60 vs. 1.40, z = 2.40, p = 0.027) (Table 3).

Table 3

Statistically significant correlation of hand changes and working ability

Parameters	Group I mean ± SD	Group II mean ± SD	z	p
Reducing time spent on the job				
Diminished muscle strenght on:				
fingers				
FPL	1.42 ± 0.67	2.50 ± 1.50	2.20	0.041#
ABD	1.08 ± 0.28	2.25 ± 1.03	3.74	0.002##
ADD	1.17 ± 0.38	2.25 ± 1.58	2.30	0.34#
hands				
EDC	1.16 ± 0.38	2.38 ± 1.68	3.31	0.004##
FDS	1.42 ± 0.67	3.00 ± 1.50	3.22	0.005##
FDP	1.50 ± 0.80	3.00 ± 1.50	2.91	0.009##
Difficulties in manual actions				
Unscrewing taps	1.25 ± 0.45	2.00 ± 1.31	1.85	0.050#
Buttoning buttons	1.16 ± 0.39	0.75 ± 0.89	2.02	0.048#
HAMIS test (fingers + hand + lower arm)	1.17 ± 1.08	8.25 ± 7.08	3.34	0.004##
Failure to meet standards				
Pain in the hands	2.25 ±0.15	3.25 ± 0.89	2.20	0.041#
Weakness in the hands	1.42 ± 0.51	2.13 ± 0.99	2.10	0.050#
Stiffness in the hands	1.83 ± 0.83	3.00 ± 1.06	2.74	0.013#
Difficulties in manual actions				
buttoning buttons	1.17 ± 0.39	1.75 ± 0.88	2.03	0.050#
Thickening of the skin (mRSS) on:				
hands	3.10 ± 1.02	3.50 ± 0.53	2.05	0.050#
HAMIS test on:				
fingers	0.67 ± 0.63	3.88 ± 3.22	2.51	0.022#
fingers + hand	1.50 ± 1.57	7.00 ± 6.67	2.61	0.018#
fingers + hand + lower arm	1.67 ± 1.35	7.50 ± 7.19	2.51	0.022#
Leaving out some of the work				
Pain in the hands	1.83 ± 0.98	3.00 ± 0.96	2.47	0.024#
Diminished range of movement:				
flexor of III finger mid.phal.	94.33 ± 10.13	69.57 ± 27.04	2.39	0.028#
Investing an extra effort				
Dissatisfaction with health status	2.60 ± 0.89	3.80 ± 0.77	2.89	0.010##
Pain in the hands and fingers	2.00 ± 1.00	3.87 ± 1.06	3.45	0.003##
Stiffness in the hands and fingers	1.40 ± 0.54	2.60 ± 1.05	2.40	0.027#

Group I – patients with no problems at work; Group II – patients with problems at work; SD – standard deviation FPL – *flexor pollicis longus*; ABD – *abductor pollicis*; ADD – *adductor pollicis*; EDC – *extensor digitorum communis*; FDS – *flexor digitorum superficialis*; FDP – *flexor digitorum profundus*; HAMIS – hand mobility u scleroderma; mRSS – modified Rodnan skin score.
***p* – statistical significance; z – value of the Mann-Whitney test; # – statistically significant; ## – statistically highly significant.**

Table 4

Statistically significant correlation of hand changes and the WAI score

Hand changes	WAI groups			χ^2_{kw}	*p*
	excellent (mean ± SD)	good (mean ± SD)	moderate (mean ± SD)		
Stiffness in the hands	1.88 ± 0.92	2.20 ± 0.83	3.00 ± 1.26	2.77	0.050#
Weakness in the hands	1.44 ± 0.52	1.40 ± 0.54	2.33 ± 1.03	3.34	0.050#
Difficulties in manual actions (unscrewing taps)	1.22 ± 0.44	1.22 ± 0.44	2.33 ± 1.36	3.83	0.042 #
Thickening of the skin (mRSS) on: fingers	3.44 ± 0.72	2.60 ± 1.14	3.67 ± 0.51	3.74	0.050#
Diminished range of motion in: thumb ABD	1.11 ± 0.33	1.40 ± 0.54	2.33 ±1.21	4.98	0.020#
thumb ADD	1.22 ± 0.44	1.20 ± 0.44	2.50 ±1.75	3.31	0.050#

mRSS – modified Rodnan skin score; ABD – *abductor pollicis*; ADD – *adductor pollicis;* SD – standard deviation.
***p* – statistical significance; χ^2kw – value of the Kruskal-Wallis test; # – statistically significant; ## – statistically highly significant.**

WAI questionnaire demonstrated that the working capacity was reduced in 11/20 (55%) of our subjects. In the group of the most serious cases (the "poor" category), there were no subjects; there were 6/20 (30%) in "moderate" category, while 5/20 (25%) of subjects were in the group with minimally reduced working capacity ("good" category).

These findings were in statistically significant correlation with our data regarding stiffness in the hands ($\chi^2 = 2,77$; $p = 0.050$), arms weakness ($\chi^2 = 3,34$; $p = 0.050$), problems with manual activities (opening the tap) ($\chi^2 = 3,83$; $p = 0.042$), followed by a thickening of the skin of the fingers on both hands ($\chi^2 = 3,74$; $p = 0.050$) and reduced mobility of thumb bilaterally (ABD - $\chi^2 = 4,98$; $p = 0.020$ and ADD - $\chi^2 = 3.31$; $p = 0.050$) (Table 4).

The degenerative changes in the small joints of the wrist and fingers (osteoarthritis/ arthrosis on DIP and PIP) were visible at X-ray images of the hands in 4/20 (20%) of our subjects. None of them had ulceration of the fingertips on hands, acroosteolysis, calcinosis or tendon friction.

Comparing other changes on hands with problems at work and with the WAI score, no statistically significant connection was found.

Comparing the capillaries, skin thickness, muscle strength and range of motion of the right and left hands, no statistically significant difference was found.

Discussion

Impaired function of the hand in SSc is of multifactorial origin and is the result of the pain, increased skin thickness, skin sclerosis, subcutaneous calcinosis, thickening of the connective tissue in the deeper layers of the dermis and muscle fascia, lesions in the bones and joints, the microvascular lesions (paroxysmal vasospasm or permanent ischemia with subsequent digital ulcerations), contractures, tendon shortening, rubs friction on tendons and reduced strength[1–4]. Significant functional disability occurs in excess of 50% of patients in first 18 months from the onset of the disease.

All mentioned elements result in reduced use of damaged hands, affecting the working ability and the quality of life.

The physical pain associated with SSc is present in all patients at different stages of the disease. The first painful sensations are due to vasoconstriction of the small arteries and arterioles of fingers and manifested as Raynaud's phenomenon (RP). Pain can occur occasionally in the attack, it is mild and lasts up to half an hour, rarely longer. It is followed by pain due to muscle, bone and joint lesions, as well as the lesions of the sensitive nerves, most often in the area of the wrist, at rest or in motion, lasting several hours and mostly medium to high intensity. Later, with the evolution of the disease, the causes of pain are also the lesions of the internal organs [15, 16].

Stisi et al. [17] have analyzed the incidence of pain induced by damage of the musculoskeletal system in 242 patients with SSc. The pain was present in 83% of patients.

According to Clements [18], pain due to changes in the locomotor system is present in 40–80% of patients with SSc, especially in the initial phase of the SSc.

In our study group, pain was present in 19/20 (95%) of the patients, with 13/20 (65%) of subjects complaining about moderate to severe pain in the hands and fingers.

mRSS is the most suitable method for assessment of the prevalence of skin lesions, which is the basis for distinguishing between limited and diffuse forms of the disease, also to assess the success of the applied therapy, and as an indicator of the disease activity and outcomes [6]. Changes in the skin significantly correlate with mRSS [19]. In the early stages of SSc, extensive skin changes correlate with severe changes on the internal organs observed later in the disease, poor prognosis and increased disability.

Sawy et al. [20] evaluated the functions of hands in 15 patients with SSc and found increased thickness of the skin and *flexor retinaculum*, thickening of inter-metacarpal area II as well as the reduced area of innervation of median nerve, which was significantly associated with reduced grip strength as well as with reduced range of motion of the fingers. They concluded that impaired hand function was mainly linked with reduced mobility and reduced hand strength.

For the purposes of this study, only mRSS values relating to the quality of the skin of fingers and hands were taken. Changes on the hands were found in 14/20 (70%) of our patients, and in more severe forms (thickened skin unattached or attached to the deeper layers) in 7/20 (35%) of them. This finding failed to show a statistically significant correlation with

working capacity. Changes on the fingers were detected in a higher percentage – 19/20 (95%), and were significantly associated with the impairment of working capacity.

Capillaroscopy provides quite accurate assessment of blood vessels condition. On the basis of this finding, it is possible to differentiate primary from secondary Raynaud's phenomenon (RP), to detect a disease before other clinical manifestations, predict the development of the disease, to assess the disease activity and early detect involvement of the internal organs in SSc [7, 21].

"Scleroderma type" capillary lesions, classified as a type II and III in patients with RP can be seen months and even years before the manifestation of other signs of disease, and represents valuable sign for the early detection and prediction of the disease development [22]. Early diagnosis of SSc so far represents the only realistic chance for successful treatment.

Analysing 3,035 capillaroscopy findings in patients with primary RP, scleroderma type lesions were registered even 6 months prior to the manifestation of other signs related to the disease [23].

The secondary RP is in most cases an introduction to one of systemic autoimmune diseases or the microcirculation disorder. In about 90% of all patients with SSc, RP is the first symptom of the disease. The characteristic of the secondary RP is pathological capillaroscopy pattern, also referred to as scleroderma pattern [21, 24].

Koenig et al. [25] announced that a high percentage of patients with RP and with capillaroscopy finding typical for SSc, with no other signs except the bloated fingers and/or arthritis, will, over time, develop diffuse form of SSc, and suggested the name "early systemic sclerosis" (eSSc).

In our study the pathological lesions of the capillaries were demonstrated in high percentage (15/17, 85%). In 6/17 (35%) of our subjects, it was type II lesion according to Maricq, which was considered to be an indicator of a mild form of SSc and better prognosis. Type III was registered in 8/17 (45%) of the patients, and type IV in a single patient, pointing towards more severe disease form with a poor prognosis. Changes in the capillaries in our patients did not show a statistically significant association with the reduction of working capacity.

Ulcerations of fingertips lead to significant functional impairment, disability and decreased life expectancy. They can be complicated with infections, gangrene and amputation. They are present in 44–60% of patients with SSc, and are associated with early RP, dcSSc, with deterioration in findings on capillaroscopy and decreased survival [26–29].

Khimidas et al. [29] analyzed the correlation of digital ulcerations with other manifestations of SSc in 938 patients and found that digital ulcers were associated with increased mRSS of hands and fingers, duration of the disease, interstitial lung disease in both dSSc and lSSc.

In our study group no digital ulcer was registered. This can be explained by the timely diagnosis of SSc in our patients, periodic monitoring in a hospital setting in order to check the current status, perform additional diagnostics or adjust medical treatment, as well as less pronounced changes in the blood vessels, which are normally considered to be a major pathophysiological mechanism for onset of ulcers.

Impaired function of the hands and fingers is manifested mainly by reduced ability of MCP joint flexion, limited extension of PIP joints, and flexion of DIP joints, decreased abduction (and soon the opposition and flexion) of the thumb, and loss of motion in the wrist (RC) in all directions. All that is accompanied by muscle hypotrophy, reduced strength, as well as the pain that can occur even after a few repetitive movements, common in everyday life and work. These changes are rarely isolated, and often present simultaneously, and affect the working activities significantly.

Decrease in muscle strength symmetrically on the extremities was confirmed by MMT in 10% of the patients [18]. Sometimes it is difficult to separate the primary myopathic weakness from the movement restrictions caused by thickening of the skin, changes in the joint near the affected muscle or surrounding tissue fibrosis. Patients with skeletal myopathy do not have worse prognosis compared to those without myopathy.

Reduced hand muscle strength was confirmed in all subjects in our study. In relation to the maximum possible maintenance of the strength (100%) the reduction ranged from 10% to 50%.

Bone and joint damages (arthralgia, arthritis, arthrosis, contractures, tendon friction rubs, tenosynovitis) are very common, they are among the first manifestations of the disease, causing considerable disability and often indicating the involvement of the internal organs [2, 18, 30–32].

Lesions in the MCP, PIP and DIP joints seen on X-ray images, are in the form of joint space narrowing, erosions, intra-articular calcification, juxta articular osteoporosis/ osteopenia and subluxation [2, 33, 34].

Avouac et al. [34] analyzed X-ray images of hands in 120 patients with SSc and found the presence of erosion in 21%, narrowing of the joint space in 28%, arthritis in 18%, demineralization in 23%, acroosteolysis in 22%, flexion contracture in 27%, and calcinosis in 23% of the cases.

In our subjects, X-ray images revealed degenerative lesions (osteoarthritis-arthrosis) in 4/20 (20%) of the patients. There were no signs of calcinosis, acroosteolysis, demineralization or arthritis.

Contractures of small finger joints are the most common joint lesions in SSc [31% according to the European Scleroderma Trials and Research (EUSTAR) Group] and are the major cause of functional disability [35].

There is no consensus on what degree of motion reduction in joints may be called the joint contracture, and therefore the prevalence of contractures found by physical examinations in the various studies varies from 26% to 56%. In spite of the slow and gradual onset, contractures may occur even in the early stages of the disease. They are result from thickening of the skin, only in the shallow layers at first, followed by connective tissue thickening in the deeper layers of the dermis and muscle fascias, peritendinous sclerosis causing tendons shortening, as well as the destruction of the joints which leads to ankylosis. Contractures of small joints of hands (MCP, DIP and PIP) are mostly flexorial (PIP),

sometimes in combination with the extension contracture (MCP) of joint (claw fist). DIP joints are less frequently affected by flexion contracture and thumbs are mainly affected by adduction contractures. They are more common in dcSSc on the dominant hand. Involvement of more than 4 joints on the same hand is a sign of poor prognosis. It is associated with interstitial lung disease, changes in the esophagus and cardiac involvement [2].

In our work, decreased range of motion in the joints of the fingers of both hands was registered in 16/20 (80%) of the patients. Most often it was in the small joints of the thumb, index finger and middle finger of both hands. The largest reduction in range of motion was 89% of the maximum.

For estimation of work ability, the primary version of WAI is used. It was established and used at the end of twentieth century by researchers from Finland [36]. The WAI is used today in Finland as a standardized questionnaire of the Finish Institute of Occupational Health (FIOH). In more than 25 countries, the WAI is accepted as a questionnaire for self-estimation of working ability, taking into consideration physical and psychological capacities of an individual [37–40].

Sandqvist at al. [41] examined the WAI in 48 patients with SSc. Thirteen patients had good or excellent WAI, 15 had moderate, and 20 had poor WAI. Patients with good WAI had less severe symptoms (pain, fatigue, impaired hand functions), better ability to adapt to tasks and needs of the workplace in relation to the patients with worse scores on the WAI questionnaire. Fatigue and decreased functionality of the hand had the greatest impact on the WAI questionnaire.

The WAI questionnaire demonstrated that the working capacity was reduced in 11/20 (55%) of our patients. Those were the patients classified in the category "moderate" and "good". There were no subjects in the group of the most serious cases, the "poor" category, i.e. where respondents expressed extremely diminished ability to work.

Lesions on the hands, which are present even in the early years of the development of SSc, are responsible for striking disability. The milder disorders present in the beginning of the disease, include occasional and brief spasms of blood vessels, mild thickening of the skin, arthralgia and occasional mild to moderate pain. At that stage of the disease, there is a limited ability to work, especially for manual jobs.

The progression of the diseases results in serious disturbances which include more frequent vascular spasms with longer duration, ulceration of the fingertips, expressed thickening of the skin, acroosteolysis, ankylosis and deformity of the joints, calcinosis, contractures, and frequent medium and severe pain. Associated with damage to internal organs, these disorders lead to complete loss of ability to work.

Our study included relatively small number of patients, so the results cannot be generalized to the whole population of patients with systemic sclerosis. However, our findings are providing solid evidence of significant connection between reduced hands function and performing working and daily activities. Further investigation on a larger population is needed to confirm our results.

Conclusion

The results obtained by examining hands impairment in patients with systemic sclerosis, showed that there were: thickened skin on the hands and fingers, reduced muscle strength and decreased joint mobility in the fingers and changes in the finger capillaries.

The results of the assessment of working ability in patients with systemic sclerosis, showed that the subjects solved their problems at work by reducing time spent on the job, by leaving out some of the work, by failure to meet standards and by investing an extra effort in work or other activities. Working capacity was reduced (through the WAI evaluation) and it belonged to categories "good" or "moderate".

There was a statistically significant correlation between altered hand functions and diminished working capacity.

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Acupuncture, autonomic nervous system and biophysical origin of acupuncture system

Akupunktura, autonomni nervni sistem i biofizičko poreklo akupunkturnog sistema

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Ključne reči:
lečenje akupunkturom; akupunkturne tačke; biofizika; nervni sistem, autonomni; fascija; kardiovaskularni sistem.

Introduction

Acupuncture (针刺, zhēncì) represents a therapeutic method of traditional Chinese medicine (TCM) that is based on stabbing needles in specific places on the skin called acupuncture points. They are located along meridians through which qì (气), vital force is running. Unity of dual principles yin (阴, yīn) and yang (阳, yáng) in organism is comprasing qì and they are reflected in the form of periodic (night-cooling and day-rising [*]) Disturbence of qì flow TCM physicians consider as a cause of disease occurence. They use acupuncture in purpose of lǐqì (理气), regulation and reparation of qì flow [1]. For good theraputic responce it is necessery to find and insert needle in acupuncture point, point of qì (气穴, qìxué). Sign of insertion needle into acupuncture point (qìxué) is local sensation of specific pain, numbness, heaviness... It is called déqì (得气). In order to find acupuncture point (qìxué), TCM physicians use old Chinese maps of acupuncture system (AS). These Chinese maps of AS (经络, jīngluò) were established about 5,200 years ago [2]. However, they are still scientifically unexplained. Last 4 decades numerous biomedical reports have not provided an agreement about determination of physical structures of AS (jīngluò) [3]. There are several histological candidates: microtubules, gap junctions, clusters of water [1, 4], collagen fibers, fascia [3], Bunghans channels (tread-like structures identified inside of the lymph channels) [1], nerves of central nervous system (CNS) [5] and autonomic nervous system (ANS) [6], etc. Due to impossibility of proving absolute physical or functional identity of AS (jīngluò) with any of these structures or with other organic systems, it is uncertain whether AS (jīngluò) and qì have anatomical analogons in classical medicine. Appropriate hypothetic biophysical model of AS (jīngluò) should give explanation of one seemingly simple phenomena: in which way inserting little and sharp needles (without injection of substance or radiation) is producing effective and fast clinical outcomes [7]. Characterization of biological structures of AS (jīngluò) would help in clarifying mechanisms of acupuncture and related techniques, as well as the development of new methods for diagnostics and therapy based on TCM principles. But, so far histological research has not identified structures that fit with anatomical locations of AS (jīngluò) presented in Chinese maps. It is possible that AS (jīngluò) is not made of physical structures, but from functional (physiological or signaling) interconnections that old Chinese were symbolically representing in form of anatomical paths (luò), the well-known acupuncture meridians. If that is so, than modeling physiological processes during therapeutic responses of acupuncture might be significant for indication on nature of AS (jīngluò). Bearing in mind that ANS is one of the most significant systems that regulates organic func-

[*] It is incoded in etymology of Chinese characters: characther for yīn contain subcharacter 月 (yuè) which means moon and character for yáng contain subcharacter 日(rì) which means sun.

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tions [8–15] it follows that characteristics of ANS exactly should be in focus of evaluation before and after acupuncture therapies. Therefore, it is first necessary to explore and summarize recent research about the influence of acupuncture on ANS. The research will speak firstly about the nature of AS (jīngluò) and significance of ANS for therapeutic effects of acupuncture [3, 6].

Influence of acupuncture on autonomic nervous system

In the review article "Acupuncture and heart rate variability" Korean researchers, Lee et al. [16] made an overview of articles from 14 databases, without restriction of year and language. Only 25 papers have passed their criteria of control randomized trials. They determinate that only 5 studies showed significant difference of effects of acupuncture on heart rate variability (HRV) in comparison with sham (placebo) acupuncture in control groups. They claim that these results are not consistent and methodologically comparable between each other. So, they conclude that (until their review research) there are no evidences that acupuncture produces significant effects on HRV, one regional sector of ANS. Since the ANS is regionally organized and regionally controlled, this report does not exclude that neural regulation of other organ systems than cardiovascular (i.e. cerebral blood flow, respiration, temperature regulation, muscle blood flow, alimentary function, renal function) is influenced by acupuncture [17, 18].

Therefore, our theoretical analysis takes in consideration papers about influence of acupuncture on ANS published from 2010 to present. In order to approach them we have choose 3 most common used web services for biomedicine topics (Science Direct, PubMed and Medline). In browsers of it, words "acupuncture" and "autonomic nervous system" were typed. We found and analyzed total of 18 papers[19–37] that were directly related on this topic. Main inclusion criteria were use of manual acupuncture in human subjects because others involve various factors like biochemical or electromagnetic influence on different organism regulation mechanisms. Thus, research of electro acupuncture, laser acupuncture, moxibution, pharmacoacupuncture and research on animals were excluded. It is possible to derive several introducing remarks: in most papers evaluation of cardiovascular autonomic functions was obtained through time and frequency analysis of HRV; short term measuring procedures of parameters of autonomic regulation were usually performed (5, 10, 15, 30 min) before, during and after one acupuncture treatment. In all papers there is significant positive effect of acupuncture at least on one parameter of cardiovascular autonomic regulation. After these insights it is possible to extract several assumptions about biophysical base of acupuncture influence on ANS.

Hypotheses about influence of acupuncture on autonomic nervous system

In single research papers about influence of acupuncture on ANS [6, 19–37] there are few detailed interpretations of obtained results in context of explanation of mechanisms of acupuncture. Thus, generalization of these various research results can hardly be reduced to a single theory. While summarizing results from reviewed papers we generalized several hypotheses that might indicate on biophysical nature of AS (jīngluò). Those are: neural hypothesis, pain stimulus, placebo effect, mechanistic model and complementary model of ANS and AS (jīngluò).

Neural hypothesis

Despite of significant AS (jīngluò) and CNS relationships [5, 38], correlation of changes in autonomic functions and electroencephalography (EEG) signals under the influence of acupuncture [39], there are no clear evidences of coincidence of AS (jīngluò) and the so called psycho-neuro-endocrine system [40]. First of all, acupuncture points and meridians (qìxué, luò) within Chinese maps are not coinciding anatomically with nerves of CNS and ANS. That was pointed on in handbooks of Serbian acupuncturologists and biomedical engineers more than 30 years ago [41, 42]. Accidently, certain acupuncture points could be found on places of neural pathways. Also, from the review of the literature [6, 19–37] there are no direct confirmations that acupuncture effects are made only through neural mechanisms. It is certain, however, that ANS, under the influence of acupuncture, optimizes involuntary functions (for example pulse, blood pressure, contractions of smoot muscles of abdomen etc). These are efferent (motor) neural mechanisms. It is not clear which mechanism is in the basis of influence of acupuncture on ANS. From positive effect of acupuncture on cardiovascular diseases Mehta et al. [32] assume that cardiovascular-specific acupuncture points are stimulating groups of III and IV afferent (sensor) autonomic neural pathways; through them neural signals are forwarded to regions in hypothalamus, midbrain and medulla that, afterwards, regulate cardiovascular system [29]. Longhurst [6] agrees with pointing on these sensor nerves in transferring acupuncture stimuli. Referring to objections that his hypothesis gave no explanation of clinically observable characteristics of AS (jīngluò) [43], Longhurst replays that while applying electroacupuncture on stomach meridian his team in Graz got results of change in sympathetic outflow that they assume to come from visceral region. This is possible because stimulated nerves are crossing in common interneural parts of medulla from which, primarily, regulation of cardiovascular system (CVS) is directed. Then, he claims that there are no evidences of existence of meridians in physical form. There are neural afferent pathways, i.e. from foot to spine that are longer than 1 meter. For laser acupuncture he claims that there are not enough publications in high quality journals [44]. However, as it was mentioned, ANS is not anatomically identified with AS (jīngluò). His explanation of afferent mechanisms of acupuncture should be explored at least through correlation of parameters of functions that are regulated by ANS and parameters of neural activities of afferent autonomic nerves. Toma et al. [21] determined in 14 subjects that acupuncture of LI4 acupuncture point in rest cause no effects on HRV and skin sympathetic neural activity (SSNA,

measured on peroneal nerve by means of intercellular electrodes); otherwise, during handgrip it gives results of lesser increase of SSNA than without acupuncture treatment. Authors of research assume that this result might be obtained because acupuncture decreases central sympathoexcitatoric effect on the skin that is usually mobilized by physical activities (exercise). Bearing in mind that LI4 is innervated by radial nerve, and that neurogram was recorded on common (peroneal) nerve, they conclude that effect of acupuncture in their experiment was systemic (non local). Yet, they admited that detailed mechanism of their result is unknown. Anyway, if future research confirms their assumptions, several questions will still left without answers: how acupuncture affects nerves in cases when they are not coinciding with places of acupuncture points? Which kind of stimuli takes place: ordinary mechanical, biochemical or something else? How are achieved fast effects of acupuncture stimulations, and how are achieved long lasting therapeutic effects, especially in diseases that are not neural? Cheng [33] thinks that if acupuncture might produce signal to brain to secret out endorphins, then it can produce signal for other neurotransmitters as well. Local effects of acupuncture on examples of muscle-skeletal disturbances, he interprets that comes from micro-damage of tissue, increased local blood circulation, mechanisms of regeneration and analgesia. Accordingly, acupuncture needles stimulate nerves in local tissue. However, ancient TCM physicians would hardly be satisfied with theoretical identifications of AS (jīngluò) and ANS. If the effects of acupuncture are reduced to neural mechanisms only, old Chinese concept of AS (jīngluò) becomes an abstract construction. In line with that, no research so far has obtained evidence for this. On the basis of the above results it is more certain that some structural-functional dependence exists between AS (jīngluò) and ANS.

Painful stimulus

In their research Kang et al. [20] determinate a statistically significant decrease of HRV and increase of skin conductance response (SCR), but without significant difference between real and sham acupuncture. Subjective feeling of pain with real acupuncture treatments was more correlated with SCR changes than with changes of HRV. In sham acupuncture (control group) there was no such correlation. Therefore, authors of research made a conclusion that acupuncture by itself does not produce real therapeutic effect. In their opinion, the therapeutic effect is more related with response of ANS on pain. Also, response of ANS during acupuncture stimulation is not necessarily reflecting subjective reaction on pain, but expecting painful stubbing as well. By means of correlation of magnetic resonance imaging (MRI) excitation of brain regions and HRV during acupuncture Beissner et al. [25] assumed that acupuncture might be specific pain stimulation. In that way pain from acupuncture might create analgesic effects and therapeutic potentials. It was concluded, from insight that stronger and more painful acupuncture stimulation was, greater decrease of heart rate was registered. Similar results were obtained by Bäcker et al. [27]. They found that significant increase of electrodermal activity was greater during more intensive acupuncture stimulation. Their explanation is that greater doses of acupuncture lead to higher pain in subjects. Then, this painful stimulus activates sympathetic ANS response and defending mechanisms. From this it could be assumed that AS (jīngluò) represents hypothetical paths through which painful stimulus is transferred to ANS. ANS reacts on pain, stress, fear, expecting the pain etc., in that way that it puts physiological functions in the best state of readiness to react on the threat or danger. Usual pain acts like an alarm for ANS to drive physiological functions in state well known as fight or flight. Contrary to this, in several research articles [19, 28, 29, 37] it was shown that epifascial acupuncture (Japanese technique of fast needling in acupuncture points) produced therapeutic effects without pain and déqì effect. In addition, effectiveness of laser acupuncture, moxibution, microwave resonant therapy and other techniques that are applied on acupuncture points without pain and déqì effect is challenging pain stimulus explanation.

Placebo effect

In several research paradigms there are results without significant difference between real and sham acupuncture [20, 21, 16]. These results are recently used as the evidence that acupuncture does not create real therapeutic effects. Instead, placebo effect might be taking place not just in sham, but also in real acupuncture. Out of 38 articles on randomized controlled trials, published until 2009, Moffet [45] found that in 22 of them there was no diference between real and sham acupuncture (in 13 studies they were equally ineffective, in 9 they were equally effective). Zheng et al. [46] claim that without patient's expectation of therapeutic effect acupuncture is nothing more than ordinary mechanical hurting. It would mean that belief and expectation of patients transform needling into therapeutic procedure. Ker et al. [47] assume that expectation and attention focused on certain points on the skin might have specific effects on neurons in region of primarily somatosensory cortex that corresponds to location of these points, even before touch or stimulation of it really happen. Cognitive perception and processing stimulation often take place in ambient of ritual relationship of physician and patient, which represents certain amount of suggestion. State of the art is the fact that acupuncture research so far is not overcoming "the placebo barrier" (conclusion of majority of review papers that effect of acupuncture is not better than placebo) [48]. Anyhow, in review article of Enk et al. [49] it was shown that there was a difference in analgesic effects of acupuncture in respect to sham acupuncture. Further- more, some researches compared acupuncture with drug therapies, but their clinical settings were of poor quality, and in contrast with acupuncture practice [48]. It turns out that trends in research are influencing on effect of sham acupuncture. In earlier research sham acupuncture was conducted with ordinary needling in non-acupuncture points. Then, minimal needling and dose of stimulations were applied. Finally, last few years the researchers use nonpenetrating needles (fake needling) in non-acupuncture points. It would be logical to expect that these changes of sham method should cause de-

creasing of its effect (placebo) during time. But, We et al. [50] gave evidence that there is an increase of sham acupuncture effect as its date of publication is closer to the present time. After reconsidering all possible factors they think that it is probably due to method of article selection for publishing in journals. Anyway, future researches have to solve the mechanisms of placebo effect; especially the relationship between expecting therapeutic effect of acupuncture (relief of symptoms), voice suggestion of doctor and autonomic placebo effect [51]. It means that experiments should be designed to contain comparison with sham acupuncture and clinical procedure without acupuncture. That is so, because some researches showed that both real and sham acupuncture could be more effective than classical therapy [45]. These insights indicate that placebo effect is something more than subjective suggestive effect or spontaneous remission. Good results made by intentionally placebo therapies are supporting this assumption [40, 52]. Ten years ago Sherman and Hickner [53] showed that many physicians in the United States from time to time prescribe or administer placebo to patients, *ie* as a supplemental treatment. Recent neurocardiological researches [52] have shed a new light on placebo since it is not reasonable any more to consider placebo as just suggestion that afterwards creates psychosomatic effect. Placebo creates clinical result, not just as a result of expectation but also by means of information influence [54]. A sort of this information influence is, primarily, not physiological, but physical. Information for therapeutic action could be coded on nano level of structural organization of biomolecules and water [54]. Hence, some questions are plausible to be asked about, like: is any expectation or any suggestion going to work without such corresponding information? Or maybe expectation and suggestion can create (incode) such information? Bearing this in mind, even if without doubt modern researches discover significant role of placebo in acupuncture mechanisms, it does not mean that acupuncture is 'placebo' method and that AS (jīngluò) does not exists. On the contrary, along with this analogy other therapeutic methods could be discredited by means of placebo effect power.

Mechanistic model

Yang et al. [55] assumed mechanistic model of AS (jīngluò) based on forwarding mechanical disturbance from needling muscle tissue through mechanoreceptors and gap (Ca^{2+}) junctions. Registered by them on MRI, sound wave (mechanistic process), artificially created with special needle on acupuncture point GB35 was propagating in longitudinal direction two times faster than in transversal. Afterwards, they investigated possibility of sound wave to activate cells of fibroblasts, that are known as mechano-sensor cells. Therefore, they recorded propagation of Ca^{2+} wave in cells fibroblasts. Certain distance ionic wave traveled for 230 s, which was much slower in respect to sound wave that activated the ionic wave. This result served them to conclude that action of acupuncture is not electrical or nerve-impulse but mechanistic in nature [55]. Thus, in their opinion qì is a mechanical wave that animate all other physiological activities in organism (the model of process). They hypothesized the muscle model as a structure of AS (jīngluò). In further, qì is manifested in blood flow through mechanical pressure wave whose origin comes from the muscle of heart and blood vessels. Their explanations are congruent with principles of TCM [55].

Fascia

Considering recent publications, fascia is one of the most actual structures that is assumed as anatomical base of AS (jīngluò). It is a membrane-like structure [3], enclosure and soft component of connective tissue that forms a continual matrix; it penetrates and surrounds all organs, muscles and nerve fibers [56]; so, it is considered as a communication network since it influences, and is under the influence of all organs, blood vessels and nerves [57]. Cytoskeleton of fascia is able to transfer mechanical forces. Applied on cytoskeleton mechanical forces produce biochemical cellular changes through mechanochemical conduction [56]. Therefore, qì is a physiological movement and activity (in general); déqì could be understood as arrival of movement, sign that fascia reacted on acupuncture stimulation. Acupuncture fixes the blockage, sustains needed movement and renovates disturbed activity of fascia. Thus, movement is not just a propagation of signal, but also a travelling of mechanical wave through meridians [55]. That is only part of the explanation, since there are evidences of propagation of light, electromagnetic and other waves through meridians [1, 38]. It was shown, on software constructed models of human body, that areas rich with tissue of fascia are closely identical by location with TCM maps of meridians and points [57]. Effective acupuncture places are identified with fascia that contains nerve endings, fibroblasts, undifferentiated mesenchymal cells and lymphocytes. Fascial anatomy is comprised of two systems: retaining one, made of undifferentiated cells of connective tissue with passive function of sustaining the nutritive ingredients and cells for functional system (yīn); and functional system, comprised of differentiated cells that have active function of maintenance of metabolic and life functions (yáng) [57]. Thus, if fascia is biological base of AS (jīngluò) it is going to be crucial to explore correlation of activities in fascia with autonomic functions (nerve signals and electroconduction) especially because fascia is highly innervated with nerves of ANS [56].

Model of complementary analogy of autonomic nervous system and acupuncture system

Software visualization of sympathovagal balance by means of electrocardiography (ECG) monitor instrumentation has patterns very congruent with rules of yīn and yáng coordination in TCM [1, 58, 59]. Sympatho-vagal balance of neurocardiology could be seen as complementary analogy with cang fu (zàngfǔ, 脏腑) concepts of TCM according to which all organs are connected through meridians of AS (jīngluò) and they relate with each other as yīn and yáng; qì has exact time of passing through each organ. Overactivity/deficit of

yīn or yáng are reflected in form of symptoms of diseases of organs/organism [1]. It is in agreement with mechanisms of sympathetic and parasympathetic influence both on circulation function of cardiovascular system [7, 12–14, 60, 61], and regulation of other organs. Activity of one is usually followed with inhibition of other. It is governed by CNS and peripheral reflex mechanisms [61]; while sympathetic (s) ANS increases, parasympathetic (p) ANS decreases physiological functions (heart rate, blood pressure). Therefore, in general terms, sANS and pANS could be modeled as yáng and yīn, respectively. In that manner, concepts of TCM has complementary analogies with concepts in neurocardiology: organic dysfunction and syndromes (证, zhèng) [62] are analogue with neurocardiological evaluation of states of ANS in various diseases; drop of qì (syndrome of qì deficit) is analogue with malfunction of autonomic regulation (very low value of total power of HRV spectra; it is used as predictor of sudden cardiac death); excess of yáng can be regarded as sympathetic predomination (high value of low frequency component of total power of HRV spectra); excess of yīn can be regarded as parasympathetic predomination (high value of high frequency component of total power of HRV spectra) [58, 62, 63] From these analogies, it turns out that qì could be functionally characterized by means of spectral parameters of HRV, where cardiovascular ANS has a role of functional mediation between AS (jīngluò) and CVS and, potentially, with other organ systems [38, 58].

Neurocardiovascular system and qì regulation

After review of researches in this paper, it should be evident that effects of acupuncture include action of ANS, CNS, endocrine system and other mechanisms. But, there are no clear suggestions about histological structures of AS (jīngluò). Based on these results it is not reasonable to identify AS (jīngluò) with ANS. Development of ANS starts in the third week of embryogenesis [64], while regulation processes are present earlier, even before formation of any organ system. Some research assume AS (jīngluò) as the first regulating and developing system of a number of synchronous, simultaneous, coordinated and precise embryonic processes [64–67]. After all, evidences of physical existence of AS (jīngluò) do exist: diseases of acupuncture system are sometimes expressed in the form of skin changes that are following lines of diseased meridians[3]. In the moment of deqì sensation, acupuncturists claim that occasional meridian might react by delineating through the skin. Also, there are experiments in which propagation of sensations across meridians was detected, as well as movement of isotopes [3]. However, detection of qì flow by means of any scientific method has not been done so far. Despite of it, qì flow is possible to experience and sense just like gravitation force is, no matter that gravitation is also not detectable by scientific methods. By TCM practice qì flow could be awarded. It serves even for intentioned regulation of heart rate and blood pressure (meditative breathing and exercise, qìgōng). Vitality of qì is basically under the influence of stress and emotions [1, 38, 68–70]. Relation of emotions and CNS modern research has confirmed without doubt. It is well known that brain contain network of neuronal system responsible for processing of emotion. It is called limbic system and it is directly correlated with regulating neural networks for heart regulation [67]. Based on this, we can derive and illustrate one hypothetic model of qì regulation that is in the base of acupuncture mechanisms (Figure 1).

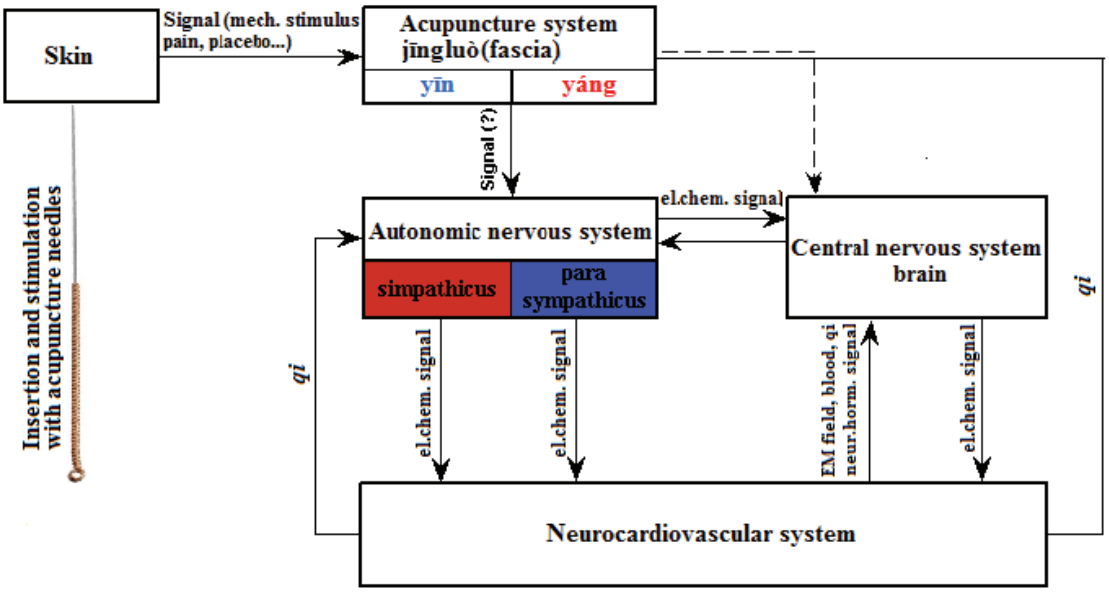

Fig. 1 – Summarizing hypothetical model of qì regulation by acupuncture: acupuncture needling and stimulations (pulling in and out, rotations of needles) creates some sort of signal (mechanical microstimuli, pain, placebo, etc.) that affects acupuncture system (fascia). From fascia, signal is forwarded through its innervated structure to autonomic nervous system (ANS). ANS sends signal to the brain that processes these informational inputs and feeds back the information to ANS in order to prepare cardiovascular system for responding to the environmental stimuli, in other words, to regulate physiological functions (e. i. blood pressure and heart rate).

Conclusion

The scope of this article was to evaluate the results of different studies on biophysical basis of AS (jīngluò). To our knowledge it represents the first theoretical survey in which hypotheses of acupuncture mechanisms were summarized from an overview of research results in articles about influence of acupuncture on ANS. However, it could not be a systematic overview nor a meta-analysis, due to the high heterogeneity of researches on related topic. Also, there was intention to analyze the topics from various points of view with different kinds of results. Mechanisms of acupuncture are not scientifically explained even in China from where acupuncture originated. More comprehensive experimental research is needed in order to evaluate all the theories of AS (jīngluò). By means of correlations of therapeutic effects of acupuncture and parameters of heart rate variability (that reflects autonomic functions) it is possible only to assume existence of functional dependence of AS and ANS. In general, our remarks are congruent with fascia as most probable candidate for acupuncture system in physical sense. Structures of fascia might receive needle inserting in form of stimuli which is by nature something among neural stimulus, pain, micromechanical movement or placebo, as overviewed in previous sections. Then, it is logically to suppose that fascia transforms these stimuli into some kind of signal (≈qì) and afterwards transfers it to ANS that lead to better regulation of physiological functions. From final perspective related with relief of disease symptoms we consider it in terms of therapeutic effects of acupuncture. In that manner, state of ANS could be significant factor of effectiveness of acupuncture therapy. Thus, besides scientific significance, this research reinforces the progress of integrative biomedical approach of two complementary medical systems - western world medicine and traditional Chinese medicine, aiming more successful diagnostics and therapy of various diseases.

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The role of physical therapy in the treatment of children with chronic functional constipation

Uloga fizikalne terapije u lečenju dece sa hroničnom funkcionalnom opstipacijom

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Ključne reči:
deca; opstipacija; elektromiografija; manometrija; fizikalna terapija, metodi; lečenje, ishod.

Introduction

Constipation is the most common functional defecation disorder affecting up to 29.6% of children with the equal prevalence in both sexes [1, 2]. In more than 90% of the children, it is functional disorder which means that it is not caused by an organic disease such as Hirschsprung's disease, neurological disorders, spinal and anal congenital abnormalities, inflammatory bowel disease, allergy, metabolic and endocrine diseases [3]. The diagnosis of functional constipation is made according to the Rome III criteria in a 4 year old child [4]. A child must experience at least 2 of the following features during the last 8 weeks: 2 or fewer defecations per week, more than one episode of fecal incontinence weekly, large stool in rectum palpable by rectal or abdominal examination, history of painful defecations, with holding behavior and occasional passing of large stools [4]. These criteria are also included in the recently published Rome IV criteria [5]. The duration of symptoms is one month instead of two months [5].

By measuring colonic transit time, 2 different types of chronic functional constipation have been identified in children: slow-transit (ST) constipation and functional fecal retention (FFR) [6]. Children with ST constipation have delayed motility in the proximal and transverse colon, in contrast to FFR, where the hold-up is in the rectum and sigmoid colon. Although the children complain of similar symptoms in both types (decreased defecation frequency, fecal incontinence, abdominal pain), their treatment involve different protocols.

Regardless of the types, the management of chronic constipation in children is based on prescribing laxatives and behavioral approaches. Long-term follow-up studies have shown that the cure rates were 50% despite this treatment [7]. Since children with FFR have contractions of the pelvic floor muscles (PFMs) or inability to relax them during defecation, physiotherapy was introduced [8].

Physical agents have mechanical and neurological effect on the colon function. Mechanical effect is reflected in stimulating peristalsis, improving fecal propulsion and tone of the abdominal muscles [9]. Neurological effects include stimulation of the parasympathetic nervous system to increase colonic motility and sympathetic stimulation, which leads to a reduction of anxiety and an increase of endogenous serotonin [10].

However, few studies have investigated the role of physiotherapy in the treatment of children with chronic functional constipation. Therefore, the aim of this review was to analyze the effects of physiotherapy interventions in childhood constipation.

Slow-transit constipation

ST constipation was first described in children with severe constipation resistant to the standard treatment, and it is now regarded as a specific disorder of colonic motility [6]. It is associated with the deficiency of substance P or vasoactive intestinal peptide in the enteric nervous system and abnormalities of the interstitial cells of Cajal [10]. The symptoms oc-

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cur early in infancy and stools are not so hard despite the long intervals between the bowel movements [11].

In these children constipation can be unrecognized, as fecal mass can not be identified on digital rectal or ultrasound examination [12]. Additional diagnostic procedures such as radiopaque marker studies or colonic scintigraphy may identify transit delay at the proximal and transverse colon [6].

Children with ST constipation respond poorly to laxatives, dietary and behavioral modifications, and toilet training. They may benefit from other treatments, such as interferential current therapy, and even surgical intervention including appendicostomy for antegrade continence enemas, colostomy, or colectomy [13].

Interferential current therapy

Interferential current therapy (IFT) utilizes two medium frequency currents which pass through the tissues simultaneously and their paths interfere with each other. At the crossover point within the tissues, this interaction gives rise to an interference current (or beat frequency), which has the characteristics of low frequency stimulation.

IFT has been used in the treatment of chronic treatment-resistant constipation and fecal incontinence in children [13]. Two electrodes, one from each channel, were placed on the anterior abdominal wall below the costal margin of the child. The other two electrodes were placed paraspinally over the muscles between T9 and L2. IFT was administrated using a carrier frequency of 4 kHz and a beat frequency sweep covering 80–120 Hz, for 20–30 min, 3 times weekly, during 4 weeks. In 7/8 children fecal incontinence disappeared, while the frequency of spontaneous defecations increased in 5/8 patients.

In the study of Yik et al. [14], it has been shown that IFT had superior effect in decreasing colonic transit time over placebo. In a randomized controlled study, 46 children were assigned to active or sham IFT during the first month of treatment (12 procedures). After 8 weeks, during a second month of treatment, all children received 12 active IFT. After the first month of treatment, transit speed was only increased in children who received active stimulation. After the second month of stimulation the number of responders treated with sham IFT in the first month was significantly increased after active stimulation.

Clarke et al. [15] investigated the quality of life in children with ST constipation before and after IFT. After a short treatment period (12 sessions for 4 weeks) of IFT, there was a significant improvement in a child self perceived quality of life.

Although the mechanism of IFT action is not fully understood, it can be attributed to activation of the sensory nerves in the skin, spinal nerves (sensory and motor T9-L2), sympathetic and parasympathetic nerves to the intestine, enteric nerves, pacemaker cells (interstitial cells of Cajal) or smooth muscle cells in the intestinal wall [12]. Also, IFT increased frequency of colonic propagating sequences and colonic motor activity on waking and after meals 2–7 months after stimulation [16].

Leong et al. [17] investigated long-term effects of IFT. IFT was applied in 39 children for 1–2 months. The procedure was continued in 15 children for additional 2 months at home. The results were evaluated after a mean follow-up period of 3.5 years. Improvement in clinical symptoms occurred in 67% of children. They reported improved rectal sensory perception and feeling the urge to defecate. Besides, fecal incontinence and laxative use decreased. These positive effects lasted more than 2 years in 33% of patients. Symptoms recurred after 6 months in 25% to 33% of children.

Functional fecal retention

The most common etiology factor is stool-with holding behavior. Changes in habits or diet, stressful events, the unavailability of toilets or delaying defecation due to lack of interest or attention, lead to stool retention. As a result of delayed defecation, rectal mucosa absorbs water from the fecal mass which becomes progressively hard and voluminous. There is a "vicious circle" in which the rectum significantly expands, resulting in the development of fecal incontinence, the loss of rectal sensation and, ultimately, loss of normal urge to defecate [18]. Besides, stool retention in the rectum provokes prolonged external anal sphincter contraction which causes pelvic floor non-relaxation during defecation [18].

In children with FFR, standard medical treatment involve education, disimpaction, prevention of reaccumulation and follow-up [18].

Education and behavioral modifications

Education about the normal bowel and anorectal function and constipation highlights importance of high-fiber diet, regular fluid intake and genital hygiene. Behavioral interventions include toilet training. Children are recommended to defecate regularly, to go to the toilet after major meals 3 times daily, to sit on a toilet seat for 5–10 minutes and try to defecate. An optimal defecation posture include an upright and forward leaning sitting position [19]. This position facilitates defecation by lengthening the anal aperture and widening the anorectal angle [19].

Disimpaction

Rectal fecal impaction is present in up to 30% of children with chronic functional constipation. Fecal disimpaction can be accomplished by oral and rectal agents. Children tolerated well rectal enemas for 3–6 days without any side effects. Polyethylene glycol was administrated orally during 3–6 days, and disimpaction occurred in up to 92% of the patients [20, 21].

Maintenance treatment

In order to prevent recurrent fecal impaction, oral laxatives in combination with behavioral modifications are prescribed over long periods. However, only 60% of children were treated successfully at 1 year follow-up. A third of the children continued to be constipated beyond puberty and a fourth in adulthood [18].

Physiotherapy interventions

The normal function of the PFMs is essential for the establishment of normal function of the bowel. The muscles of the pelvic floor are part of the abdominal capsule that surrounds the abdominal and pelvic organs [22]. The structures that form this capsule are the diaphragm, lower abdominal muscles and PFMs. These muscles act synergistically [23]. Their relaxation is essential during defecation. Otherwise, their contraction during defecation leads to the development of constipation.

More than 50% of children with FFR have abnormal defecation [11]. They contract the external anal sphincter and *musculus puborectalis* during defecation [11]. This form of abnormal defecation is considered to be learned resulting from the habit of delaying defecations.

Physical therapy starts with the education and behavioral interventions including an adequate position during defecation, diaphragmatic breathing exercises, pelvic floor exercises with or without biofeedback, in order to educate a child to relax the external anal sphincter and the PFMs during defecation. In refractory cases, even botulinum toxin injections are administrated into the external anal sphincter [24].

Diaphragmatic breathing exercises

Diaphragmatic breathing exercises (DBE) are applied in order to teach children the abdominal and PFMs relaxation. During defecation, it is important that the lower abdominal muscles *(m. transversus abdominis* and *m. obliquus internus abdominis*) and the PFMs are relaxed as they act synergistically [22]. In diaphragmatic breathing, during inspiration, the diaphragm moves caudally and the abdominal organs are pushed forward. The anterior abdominal wall bulges outward and relaxes. It has been shown that this action improves defecation [23]. Holding the abdomen in the bulged position increases intraabdominal pressure without an increase in external anal sphincter activity [22].

In the study of Zivkovic et al. [25], children with voiding dysfunction and chronic constipation were assigned DBE and PFM retraining. DBE were practiced in supine, both side lying and sitting positions in front of the mirror. Children were watching the anterior abdominal wall bulging during inspiration and they were asked to repeat the same exercise on the toilet seat and try to initiate defecation. These exercise were practiced at the clinic for 2 weeks, and then were continued daily at home for 6 months. After 6 months follow-up period, constipation was cured in all the patients.

Silva and Motta [9] combined DBEs with abdominal muscle training, abdominal massage and laxatives in the treatment of children with chronic functional constipation. The children had 12 individual sessions (2 weekly) at the hospital. The results were compared with the patients who had only laxatives. After 6 weeks of the therapy, the frequency of bowel movements was significantly higher in the physiotherapy group compared to children treated with laxatives only.

Pelvic floor exercises and biofeedback

The aim of the pelvic floor exercises is to increase children's awareness of their PFM function. Children are thought to contract and relax the PFMs at will. To improve the voluntary control of the PFMs, pelvic floor exercises are usually combined with biofeedback. It can be tactile (palpation of the pelvic floor or *m. transversus abdominis*), electromyography (EMG), and anorectal manometry. During EMG biofeedback, anal or perineal probe electrodes are used for the registration of muscle activity of the pelvic floor. It is displayed in the form of the curve which a child can watch on the monitor of a device. During PFM contraction, the curve is rising, while in the relaxation it is descending. In this way, by watching, a child is aware of the degree of contraction or relaxation of the PFMs.

The significance of biofeedback training in constipated children remains controversial. Some studies have reported positive effects of biofeedback and the success was attributed to restoration of normal defecation pattern [26–28]. In the study of Kajbafzadeh et al. [26], children with chronic constipation and/or fecal incontinence and voiding dysfunction were assigned pelvic floor exercises and biofeedback using animated computer games. The exercises consisted of 10 seconds contractions followed by 30 seconds relaxation period. Biofeedback therapy was performed twice weekly. All the children with fecal incontinence and 68% of patients with constipation were symptom-free within 6 months and 1 year after treatment.

Manometry biofeedback attempts to improve rectal sensory perception. Sensory training involves inducing intrarectal pressure using a balloon feedback device. A manometric balloon probe is inserted into the rectum, and the balloon is inflated with air to simulate the presence of feces. Defecation dynamics is studied by observing the ability of a child to expel the intrarectal balloon. A child is asked to contract the abdominal muscles and to relax the pelvic floor during an attempt to expel the balloon. A normal response occurred when the anal sphincter pressure decreased during straining. A paradoxical anal response was characterized by increased anal sphincter pressure during the defecation maneuver. In the study of van der Plas et al. [29], additional training using manometry did not result in higher success rates compared to conventional therapy in chronically constipated children. Similar results were obtained in the study of van Ginkel et al. [30].

In the recently published study, effectiveness of the pelvic physiotherapy was compared with standard medical care in children with functional constipation [8]. Pelvic physiotherapy included balance and stability exercises, abdominal breathing, and PFM exercises. Myofeedback and rectal balloon training were assigned only in the children with dysfunctional PFMs. The treatment period lasted 6 months, and children had maximum 6 sessions. The treatment was effective in 92% of children treated with physical therapy compared to 63% of children who received standard medical care. Also, in the first group, more children stopped using laxatives.

Zivkovic et al. [31] combined IFT with DBEs and behavioral modifications in the treatment of children with chronic constipation and bladder dysfunction who failed primary ca-

re interventions. Children were divided into 3 groups. Group A underwent IFT with DBE and behavioral modifications. Group B had DBE and behavioral modifications, while group C received only behavioral modifications. IFT was administrated to the abdomen, 20 min, five times weekly, during two weeks at the clinic, using a carrier frequency of 4 kHz, and a beat frequency sweep covering 80–120 Hz. DBE were practiced for 2 weeks at the clinic, and were continued at home for 1 month. After 6 weeks of the treatment, the results were compared among the groups. Significant improvement in defecation frequency and fecal incontinence was noticed only in the patients with the additional IFT.

Abdominal massage

Abdominal massage is performed using clockwise movements over the abdomen, starting from the ascending colon and moving toward the sigmoid colon. The positive effects include increase in intraabdominal pressure and rectal loading, stimulation of colonic movements, reduction of constipation symptoms and pain [9].

Conclusion

Chronic constipation is a common, frustrating and time consuming problem in childhood. Despite the standard medical treatment, 50% of children continued to be constipated after a five-year follow-up. Since dysfunction of the PFMs is the major reason for FFR in children, physiotherapy interventions might play an important role to increase the success rate. They include diaphragmatic breathing, pelvic floor exercises with or without biofeedback to teach children awareness, proper muscle function and relaxation during defecation. Although the mechanism of interferential current action is not completely understood, positive effects include increase in defecation frequency, improvement in rectal sensory perception, reduction in fecal incontinence and abdominal pain. As these effects might last up to 2 years, interferential currents are considered to be effective modality in the management of chronic functional constipation in children.

New, randomized, controlled clinical trials are needed to confirm the promising role of physical modalities in the treatment of constipated pediatric patients.

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CASE REPORTS

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Brain histiocytosis with precocious puberty and growth hormone deficiency at early childhood – A case report

Histiocitoza moždanog tkiva sa preranim pubertetom i deficitom hormona rasta u ranom detinjstvu

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Abstract

Introduction. Langerhans Cell Histiocytosis (LCH) is a rare chronic granulomatous, usually multisystem disease of elusive etiology, with peak incidence in early childhood and slow progressing course. Isolated brain histiocytosis is a very rare condition and neurological finding does not correlate with the extent of space-occupying anatomical lesions and degenerative changes. **Case report.** A girl, age 2.5 years was presented with diabetes insipidus and nearly fatal full spectrum isolated brain histiocytosis. Brain magnetic resonance imaging (MRI) showed multiple nodules with perifocal edema, the most prominent in the projection of the hypothalamus/pituitary and the stalk and in the region of the pineal gland. Identical nodules were present in both caudate nucleus and putamen, left insular subcortex, both temporal lobes, tegmental area of the midbrain, central part of pons and medulla, both cerebellar hemispheres and leptomeningeal membranes. The pattern resembled snow balls and flakes. Biopsy showed positivity for vimentin, S-100, CD-68 and CD1a markers. Treatment protocol LCH-III was not successful and a salvage treatment was refused by parents. She appeared again at the age of 7 with growth deceleration and fully developed precocious puberty. The control MRI of the brain revealed similar nodules in certain regression. Due to central precocious puberty, treatment with luteinizing hormone–releasing hormone (LH-RH) analogue was introduced. School performance was mediocre with cocktail-party effect behavior and slower speech. **Conclusion.** Brain histiocytosis is potentially fatal disease with chronic, variable, slowly progressive course and unpredictable responses to treatment protocols.

Key words:
histocytosis; brain; diagnosis; diabetes insipidus; drug therapy; treatment outcome.

Apstrakt

Uvod. Histiocitoza Langerhans-ovih ćelija (LCH) je retko hronično granulomatozno multisitemsko oboljenje sporog toka i nejasne etiologije, sa najvišom incidencom u ranom detinjstvu. Izolovana histiocitoza moždanog tkiva je vrlo retko stanje, a neurološki nalaz ne korelira sa obimom anatomskih lezija i degenerativnih promena. **Prikaz bolesnika.** Opisana je devojčica uzrasta dve i po godine sa insipidnim dijabetesom, punim spektrom histiocitoze moždanog tkiva i skoro fatalnim ishodom. Magnetna rezonanca (MR) je pokazala multiple noduluse sa perifokalnim edemom u regiji hipotalamusa, stalka hipofize i pinealnoj žlezdi. Slične promene nađene su u *nucleus caudatus*-u i putamenu, levom insularnom korteksu, oba temporalna režnja, tegmentnom delu mezencefalona, medijalnom delu ponsa i medule, obe cerebelarne hemisfere i na leptomeningama, dajući sliku snežnih grudvi i pahuljica. Biopsija je pokazala pozitivitet na vimentin, S-100, CD-68 i CD1a markere. Terapijski protokol LCH-III nije dao povoljan rezultat, a *salvage* terapiju su roditelji odbili. Devojčica se ponovo javila na pregled u uzrastu od sedam godina sa deceleracijom rasta i preranim pubertetom, kada je uvedena terapija analogom "rilizing" hormona luteinizirajućeg hormona (LH-RH). Kontrolni MR snimak endokranijuma pokazao je slične noduluse u diskretnoj regresiji. Uspeh u školi je bio osrednji, govor usporen i ponašanje ekstrovertno. **Zaključak.** Histiocitoza mozga je potencijalno fatalno oboljenje hroničnog, varijabilnog, sporo progredirajućeg toka i nepredvidivog odgovora na terapijske protokole.

Ključne reči:
histocitoza; mozak; dijagnoza; dijabetes inspidus; lečenje lekovima; lečenje, ishod.

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Introduction

Langerhans Cell Histiocytosis (LCH) is a rare chronic granulomatous, usually multisystem disease of elusive etiology, most commonly affecting the bone (skull, longitudinal bones, spine), bone marrow, skin, liver, lungs and infrequently salivary glands (parotid), thyroid gland [1], gastrointestinal tract (colon, duodenum) and brain and can occur at any age, with peak incidence in early childhood. The annual incidence in children under 10–15 years is 0.2–2 *per* 100,000 children. Granulomas are composed of immature histiocytes, lymphocytes, giant cells and eosinophils. It has a slow progressing course. Isolated brain histiocytosis is a very rare condition and neurological finding does not correlate with the extent of space-occupying anatomical lesions and degenerative changes. Magnetic resonance imaging (MRI) and histopathology show changes of brain histiocytosis into three patterns [2] – infiltration of the hypothalamic-pituitary region [3], neurodegenerative changes [4, 5] in cerebellum and basal ganglia and extraaxial lesions in the meninges, choroid plexus and pineal gland. Neurological deterioration includes reflex abnormalities, gait disturbance, ataxia, dysarthria, dysdiadochokinesis, nystagmus, seizures, spastic paresis or plegia, behavioral disturbances, poor concentration, memory and attention deficit, cognitive defects, mental retardation and psychiatric disorders. When the disease is detected at an early age, severe neurological consequences may occur in early adulthood, favoring its slow progression, possible arrests and the reactivations in the course and requirement of large devastation of axonal mass (threshold), that brain cannot compensate. Endocrine disorder triad includes pituitary gland – diabetes insipidus (in 50% of cases), precocious puberty and growth hormone deficiency [6], although secondary hypothyroidism, hypogonadism and hyperprolactinemia can occur.

Differential diagnosis of brain histiocytosis covers tuberculosis, sarcoidosis, Wegener's disease (granulomatosis with polyangiitis), *cysticercosis*, *coccidiomycosis*, *cryptococcosis*, cerebrotendinous xanthomatosis, Rosai-Dorfman disease (non-progressive and self-limited sinus histiocytosis with massive lymphadenopathy) [7], Erdheim-Chester disease (polyostotic sclerosing histiocytosis) and Machado-Joseph-Azorean disease (spinocerebellar ataxia).

A subset of cases exhibit somatic activating mutations in the BRAF proto-oncogene (responsible for a protein-kinase) [8].

Causal therapy is not known – corticosteroids (prednisolone), cytostatics passing blood-brain barrier (vinblastine), cyclosporine, irradiation, retinoic acid, myelosuppressive purine analog cladribine (2-chlorodeoxyadenosine), indomethacin, immunoglobulins [9] and melatonin were applied with insufficient success. Some hope gives treatment with kinase inhibitors (imatinib, sorafenib, vemurafenib) [10, 11].

Case report

A girl was presented with nearly fatal full spectrum isolated brain histiocytosis, growth deceleration and precocious puberty. She was admitted because of polyuria-polydipsia (urine volume more than 5L/24h) at the age of 2.5 years in generally good condition, except somnolence, mild dehydration, signs of hypermobility syndrome (general laxity) and imperfect dentinogenesis. She was afebrile, heart rate 72/min, respiration rate 16/min, blood pressure 100/70 mmHg. Body weight (BW) 16 kg (90 percentile), body hight (BH) 97.4 cm (97 percentile) which was congruent with familiar tall stature; body mass index (BMI) 17.02 kg/ m^2 (50 percentile).

Patient's perinatal history was unremarkable and psychomotor development uneventful. Four months ago she had varicella and afterwards was treated for pneumonia and pericarditis. Family history was not significant.

Previous head trauma or operation (anesthesia) were immediately ruled out as a possible cause of diabetes insipidus. Renal insufficiency and diabetes mellitus were excluded quickly. There were no bone involvement and no signs of Leterrer-Siwe or Hand-Schuller-Christian histiocytosis (without exophthalmos, skin rash or "geographic skull" on X-ray) [12]. Ophthalmologist did not find papilledema at initial presentation.

Overnight water deprivation test was inconclusive between psychogenic polydipsia and partial diabetes insipidus – urine specific gravity was around 1.010 (400 mOsmol/kg) at the end of the test. Decision was made to introduce replacement treatment with intranasal desmopressin (DDAVP) 5–10 mcg daily. The fluid intake was reduced to 1.5 L/24h. She was temporarily discharged but soon hospitalized at a local hospital for vomiting and somnolence (possible water intoxication due to DDAVP overdose since sodium was low – 128 mmol/L). Upon cessation of DDAVP and fluid intake reduction, urine specific gravity raised to 1.030 (1,200 mOsmol/kg), but 36 hours later polyuric-polydipsic syndrome developed again and DDAVP had to be given.

MR angiography, imaging and spectroscopy of endocranium (sagittal T1-weighted, transversal and coronal T2-weighted tomograms), accompanied with triplanar postcontrast study and detailed examination of the pituitary gland revealed multiple axial and leptomeningeal soft tissue nodules with perifocal edema, the most prominent in the medial line – in the projection of the hypothalamus/pituitary and the stalk (maximum diameter of 20 mm) and in the region of the pineal gland (15 mm) without cystic component and without calcifications. Identical intraaxial nodules were present in both caudate nucleus and putamen (8–12 mm), and in the left insular subcortex (17 mm) and in both temporal lobes (predominantly left, generating intraaxial edema). In the midbrain, one nodule in the left tegmental area was spotted, also one in the central part of pons and medulla with diameter of 3.5 mm.

In both cerebellar hemispheres were multiple nodules with diameters of 2–12 mm. At all leptomeningeal membranes (in the depths of sulci) multiple nodules of identical characteristics and genesis were found, corresponding to massive cerebrospinal fluid dissemination of the basic process; nodules generated parenchymal edema, both supra- and infratentorially. In the displayed myelin C-segment there were three micronodules with consecutive edema. Bright spot signal of the posterior pituitary gland was lost.

In conclusion, MRI showed multiple intraaxial and leptomeningeal nodules with perifocal soft tissue edema, most pronounced in the midline, and the projection of the hypothalamus, a tripod and the pineal gland. The presence of micronodules at all leptomeninges and in the projection of the displayed myelin C-segment might indicate cerebrospinal fluid dissemination of the underlying disease. The pattern resembled snow balls and flakes (Figure 1).

Disseminated nodules seen at MRI and mother's subsequent statement that she was treated for tuberculosis in the pre-conception period, tuberculosis, sarcoidosis, histiocytosis, germ-cell tumor (germinoma), Wegener granulomatosis, toxoplasmosis, neuroborreliosis, cysticercosis, cryptococcosis and coccidiomycosis came into consideration.

Cerebrospinal fluid (obtained by lumbar puncture) was under normal pressure, clear and with normal cytology, with elevated proteinorachia (0.83 g/L) and normal glycorachia and chloride level. Staining and culture techniques excluded presence of *Mycobacterium tuberculosis* in cerebrospinal fluid and gastrolavate; polymerase chain reaction (PCR) was not performed. Chest x-ray was normal and ultrasound of thorax revealed a tiny layer effusion in the left phrenocostal sinus.

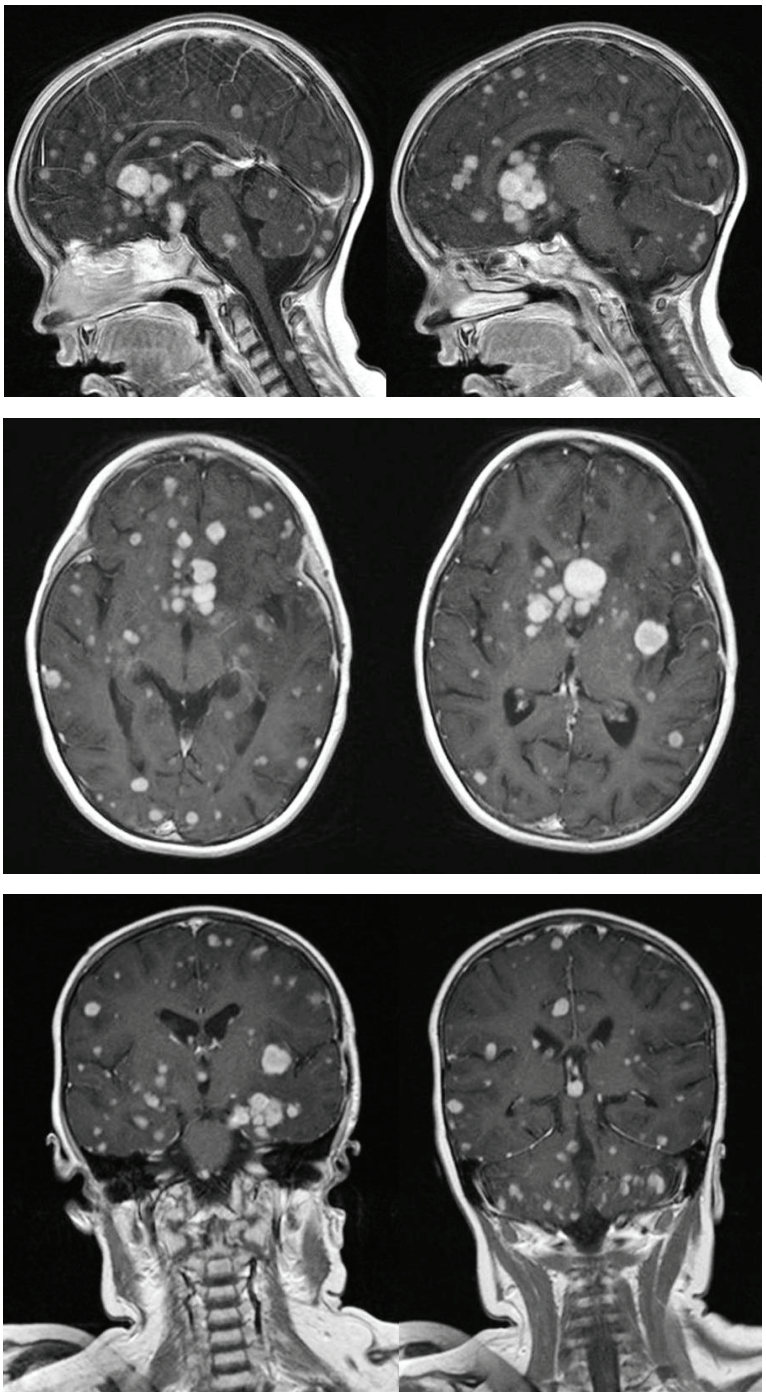

Fig. 1 – Brain histiocytosis: a) - sagittal magnetic resonance (MR) slices; b) axial MR slices; c) coronal MR slices.

Other mentioned pathology was also soon excluded. There were no signs of multiple pituitary deficiency – cortisol rhythm was normal (587.6–665 nmol/L in the morning and 148.3 nmol/L in the afternoon), as well as T3, T4, TSH and her height was initially adequate.

C-reactive peptide (CRP) was in the normal reference range, erythrocyte sedimentation rate (ESR) was 20, white blood cells (WBC) 8.0×10^9/L, red blood cells RBC 4.57×10^{12}/L, hemoglobin 127 g/L, hematocrit 34.9%, platelets 464×10^9 /L, fibrinogen 3.89 g/L, glycemia 5.03 mmol/L, sodium 139–145 mmol / L, potassium 4.4 mmol / L, pH 7.47 and urine specific gravity 1.010 (400 mOsmol/kg). Angiotensin-converting enzyme (ACE) activity was 526.6 nkat/L. Total serum proteins, calcium, phosphates, and alkaline phosphatase activity were in the normal range.

Tumor markers were repeatedly in the reference range: alpha-fetoprotein 4.6 IU/mL, β-HCG < 0.1 IU/L and lactate dehydrogenase, human immunodeficiency virus, hepatitis B antigen, antibodies against hepatitis C virus (LDH, HIV, HbsAg, antiHCV, respectively), direct and indirect Coombs test were negative. While C3, C4, total IgA and IgM had normal values, the total IgG was decreased 3.85 g/L (ref. range 5–13 g/L). Antinuclear, antimitochondrial, antiparietal, antismooth muscle and anticardiac antibodies were all negative. Ultrasonographic finding of the abdomen and electrocardiography (ECG) were without pathology and bone marrow biopsy revealed common pattern.

The patient underwent brain biopsy of yellowish nodules. Histopathology revealed histiocytic infiltration and immunohistochemistry proved positivity for vimentin, S-100, CD-68 and CD1a markers; *Mycobacterium* was not spotted.

Appropriate treatment protocol LCH-III (prednisolone and vinblastine) was started [13] and the child was much better soon after. Since the longterm response to the protocol was not as expected (the condition of the child deteriorated gradually with seizures, respiratory arrest, anisocoria, cyanosis and papilledema), a salvage treatment with cladribine [14] was proposed but refused by parents. After discharge, the contact was lost for a few years (parents referred to a religious pilgrimage) and the child appeared again at the age of 7 years in better condition, with BH 119.5 cm (50 percentile) that suggested growth deceleration, but with signs of fully developed precocious puberty (breasts stage 4 and recently occurred menarche), advanced bone age of 11 years, low insulin like growth factor (IGF)-1 and predictive height 135 cm only. She complained of dry mouth (anti-Ro/SSA and anti-La/SSB antibodies for Sjögren syndrome were negative).

The control MRI of the brain revealed similar nodules in certain regression. Due to central precocious puberty, treatment with LH-RH analogue was introduced. School performance is mediocre with "cocktail-party effect" behavior and slower speech.

Discussion

LCH is a rare chronic granulomatous multisystem disease of enigmatic etiology, most commonly affecting the bone, with peak incidence in early childhood. Extraosseaous involvement is rare [7], isolated brain histocytosis particularly. The most common cerebral location is the circumventricular organ [2] – pineal-hypothylamic-neurohypophyseal complex, then cerebellum, with headaches, seizures and diabetes insipidus as the main clinical manifestations, while growth hormone dificiency is the second most frequent endocrinopathy (up to 10%) [2]. It has infiltrative, space-occupying or degenerative character in brain tissue with cosequent cognitive and attention disorders [2–5]. This patient had the full clinical spectrum of brain tissue histiocytosis. Extensive pathological MRI pattern did not correlate with feeble neurological finding.

Conclusion

Brain histiocytosis is rare and potentially fatal disease with variable chronic course and slow progression. Causal therapy is not known and outcome is unpredictable.

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CASE REPORT

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Acute promyelocytic leukemia lacking t(15;17): molecular evidence of atypical PML/RAR-α transcriptional variant by gene sequencing

Akutna promijelocitna leukemija bez t(15;17): molekularni dokazi atipične PML/RAR-α transkripcione varijante genskim sekvenciranjem

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Abstract

Introduction. The accurate diagnosis of acute promyelocytic leukemia (APL), not only on the morphological and clinical, but also on the molecular level, is very important for application of targeted therapies. **Case report.** A 62-year-old woman presented with APL. By using conventional cytogenetic analysis as well as applying the fluorescence *in situ* hybridization (FISH) analysis it has not been possible to confirm the presence of t(15;17) in the presented patient. Using reverse transcriptase polymerase chain reaction (RT-PCR) two atypical promyelotic leukemia/retinoic acid receptor alpha (PML/RAR-α) fusion transcripts were identified. Both detected transcripts were isoforms. The larger transcript was in-frame, coding for functional aberrant PML/RAR-α protein, while the shorter transcript was an out-of-frame. **Conclusion.** Our study highlights the need for the application of molecular methodology in daily clinical practice. Precise characterization of PML/RAR-α fusion transcript creates a basis for identifying rare individual cases that require special caution when treating such patients. To our knowledge this is only the fifth case of atypical PML/RAR-α transcript containing full PML exon 7a, and among them the only one that was cytogenetically cryptic and FISH negative. All of the herein presented cases had lethal outcome. Therefore, our findings with the additional review of the literature, emphasizes the importance of detailed identification of atypical PML/RAR-α fusions, not only for the purpose of knowing their role in leukemogenesis, but also for the assessment of the impact that they can have on the outcome of the treatment.

Key words:
diagnosis; in situ hibridization, fluorescence; leukemia, promyelocytic, acute; molecular biology; reverse transcriptase polymerase chain reaction.

Apstrakt

Uvod. Precizno dijagnostikovanje akutne promijelocitne leukemije (APL), ne samo na osnovu morfoloških i kliničkih parametara, već i na molekularnom nivou, veoma je važno radi primene adekvatne ciljane terapije. **Prikaz bolesnika.** Prikazali smo bolesnicu, staru 62 godine, sa dijagnozom APL. Primenom standardne citogenetičke analize, kao i primenom fluorescentne *in situ* hibridizacije (FISH), nije bilo potvrđeno prisustvo t(15;17) kod opisane bolesnice. Primenom metode reverzna transkriptaza-lančana reakcija polimeraze (RT-PCR), identifikovana su dva atipična *promyelotic leukemia/retinoic acid receptor alpha* (PML/RAR-α) fuziona transkripta. Oba transkripta su predstavljala izoforme. Duži transkript je zadržao "okvir čitanja" i kodirao je funkcionalan PML/RAR-α aberantni protein, dok je kraći transkript bio van "okvira čitanja". **Zaključak.** Naša studija ukazuje na potrebu za primenom molekularne metodologije u svakodnevnoj kliničkoj praksi. Precizna karakterizacija PML/RAR-α fuzionih transkipta čini osnovu za identifikovanje retkih bolesnika čije lečenje zahteva dodatni oprez. Prema našim saznanjima, ovo je tek peti slučaj opisanog atipičnog PML/RAR-α transkripta koji u sebi sadrži celokupan PML egzon 7a, a među njima jedini koji se nije mogao detektovati primenom citogenetičke i FISH analize. Svi ovde predstavljeni slučajevi su imali smrtni ishod. Zbog toga, naši rezulatati, zajedno sa sličnim slučajevima opisanim u literaturi, naglašavaju

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značaj detaljne identifikacije atipičnih PML/RAR-α fuzija, ne samo u svrhu prepoznavanja njihove uloge u procesu leukemogeneze, već i u smislu procene njihovog uticaja na ishod lečenja.

Ključne reči:
dijagnoza; hibridizacija in situ, fluorescentna; leukemija, promijelocitna, akutna; biologija, molekularna; polimeraza, reakcija stvaranja lanaca, reverzna transkripcija.

Introduction

Acute promyelocytic leukemia (APL) is a well-defined clinical-biological entity of acute myeloid leukemia, characterized by unique morphology of leukemic cells and the specific t(15;17), present in approximately 80% of APL cases. However, this translocation is never found in other subtypes of acute myeloid leukemia (AML), except in rare blasticpromyelocytic leukemic transformation of a chronic myelogenous leukemia [1].

The t(15;17) (q22;q21) fuses the promyelocytic leukemia (PML) gene on chromosome 15 encoding a transcription factor, with the retinoic acid receptor alpha (RAR-α) gene located on chromosome 17, a member of a steroid hormone nuclear receptor family that is important for the regulation of both normal and malignant cellular differentiation and proliferation. The breakpoints in RAR-α gene are always located in intron 2, while different breakpoint cluster regions (bcr) were identified in PML gene. PML breakpoint bcr1 in intron 6 and bcr3 in intron 3, give rise to two different isoforms of PML/RAR-α fusion protein: long, L-isoform (bcr1) is present in about 55% of APL patients, and short, S- isoform (bcr3) is found in about 40% of patients [2, 3]. The rarest is variable, V-isoform, with the breakpoint bcr2 located in PML exon 6 [2–4].

The PML/RAR-α fusion protein has numerous functions in the process of leukemogenesis, but it also mediates response to all-trans retinoid acid (ATRA) therapy treatment [5]. Its oncogenic action, PML/RAR-α fusion protein expresses through disruption of both RAR-α and PML pathways [6, 7]. PML/RAR-α exhibits its dominant negative impact on wild type RAR-α function as a transcriptional factor. It has been suggested that deregulation of the genes that are under RAR-α control lead to a blockage in myeloid differentiation. PML/RAR-α acts as a dominant negative PML mutant. It has the ability to interact with wild type PML causing impaired functioning of PML in programed cell death and genomic stability [8, 9].

It has been known that different PML/RAR-α transcripts can have different prognostic significance. In all 3 most common isoforms, in the in-frame fusion transcripts, the RAR-α part of the PML/RAR-α fusion retains exons 3 to 9, therefore preserving DNA-binding and retinoid acid (RA) ligand binding domain. The RA ligand binding domain is very important for the sensitivity to ATRA treatment. More precisely, it was shown that the expression of PML/RAR-α fusion gene is required for the sensitivity to ATRA treatment [10, 11].

There are several reports describing different response to therapy depending on the type of the PML/RAR-α fusion transcript variants [4, 12–17]. It appears that different parts of PML genes that have been preserved and incorporated into various PML/RAR-α fusion transcripts have a unique impact on the response to therapy.

Here, we report an interesting APL case with cytogenetically cryptic and fluorescence *in situ* hybridization (FISH)-negative PML/RAR-α rearrangement, and a very aggressive course of the disease. We also present the results of RT-PCR and sequencing analysis of the two unusual PML/RAR-α transcripts found in this patient, both containing insertion of exon 7a, and the review of the previously published studies with similar findings.

Case report

A 62-year-old woman was admitted in February 2015 due to widespread muco-cutanous bleeding of two-week duration. After a brisk hematological work-up a diagnosis of intermediate risk APL was made. Namely, her complete blood count was as follows: hemoglobin level 75g/L, WBC count 4.9×10^9/L with 12% blasts and 83% promyelocytes in her leucocyte differential formula, and platelet count of 20×10^9/L. Bone marrow aspirate showed 90% of hypergranular blasts expressing the following markers: myeloperoxidase, CD13 and CD33. However, CD34 and HLA-DR were not expressed on blast cells. Her hemostasis was highly indicative of coagulopathy: fibrinogen 0.9 g/L (reference range: 2–4 g/L); prothrombin time (PT) (Quick) 40% (reference range: 75–120%), activated partial thromboplastin time (APTT) 26.5 s (reference range: 27–35 s); D-dimer 54 mg/L (normal value < 0.5 mg/L).

Cytogenetic analysis showed normal karyotype, 46,XX [20]. Interphase and metaphase FISH studies were performed on bone marrow cytogenetic specimens which were previously used for karyotype analysis. Detection of PML/RAR-α and RAR-α/PML fusion genes were performed using the DF SureFISH® 15q24.1 probe to label PML together with the DF SureFISH® 17q21.2 probe to label RAR-α (Agilent Technologies®, Ceder Creek, TX, USA). FISH analysis detected the presence of normal karyotype in all analyzed cells.

The patient was immediately started with ATRA (45 mg/m^2/day). Prednisone (0.5 mg/kg/day) was given to prevent differentiation syndrome. Despite an intensive blood product support the patient expired 4 h after admission due to central nervous system (CNS) bleeding.

For the reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing analyses total RNA was isolated from bone marrow aspirate using Trizol reagent (Invitrogen, USA). Reverse transcription was performed using 1 μg of total RNA, random hexamers and reverse transcriptase (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems) in a total volume of 20 μL, according to the manufacturer's instructions.

For the detection of PML/RAR-α transcript we applied standardized RT-PCR method [18]. Usage of this method enabled us to detect the most common PML/RAR-α transcripts due to the right combination of primers in both first and second (nested) PCR cycles. In parallel with the patient sample the adequate positive, negative and the water control were amplified. Electrophoresis of the PCR analysis was performed on 2% agarose gel stained with ethidium bromide, and visualized on an ultraviolet transilluminator.

The PCR products were cut-out of the gel and purified using QIAquick® Gel Extraction Kit (Qiagen, Germany) and were directly sequenced on ABI 3130 Genetic Analyzer (Applied Biosystems, USA). The primers used for the sequencing (forward and reverse) were the same one used in the RT-PCR analyses.

The classical cytogenetic analyses revealed 46,XX karyotype in all 20 metaphase cells analyzed. Also, the FISH analyses using dual fusion probes for PML and RAR-α genes, indicated signals consistent with normal signal pattern.

In the RT-PCR analyses using primers for the detection of bcr1/bcr2 fusion transcripts (PML-A1 located in exon 5 combined with RAR-α-B primer located in exon 3) yielded two products that were different from typical bcr1 isoform (Figure 1). Two unusually long, but very faint bands were detected in the amplification reaction using primers for bcr3 isoform (PML-A2 primer located in exon 3, in combination with RAR-α-B) (Figure 1). It was evident that it was a case of bcr2 isoform, so we performed sequencing analysis of both bands.

Sequencing analysis of the longer and dominant band showed that it was 690 bp long. Namely, in our sequencing analyses using PML-A1 as a forward primer, i.e. RAR-α-B as a reverse primer, we detected that in addition to complete exon 6 found in typical bcr1 isoform (381 bp long), there was an addition of complete exon 7a (53 bp), a part of the intron 7 (also called exon 7b) in a form of insertion of 229 bp, insertion of 27 bp that is a part of RAR-α gene intron 2, and then RAR-α exon 3 where the RAR-α-B primer is situated (Figure 2). In this fusion transcript the open reading frame (ORF) was maintained.

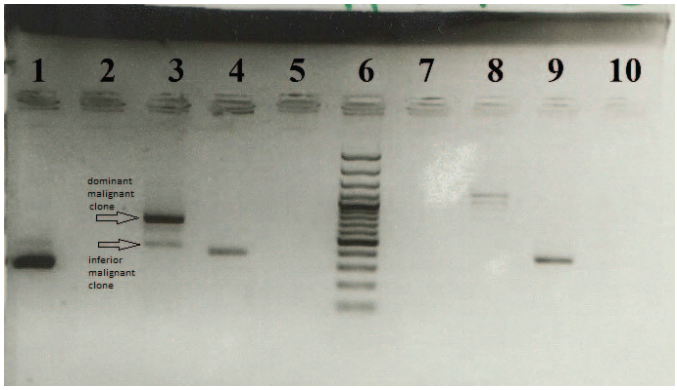

Fig. 1 – Electrophoresis of products of reverse transcriptase polymerase chain reaction (RT-PCR) analysis (1st round). Lane 1 – PCR amplification product for the housekeeping gene (ABL gene); Lanes 2 – 5 represent PCR amplification using promyelocytic leukemia (PML)-A1/retinoic acid receptor (RAR)-α-B primers: Lane 2 – negative control, Lane 3 – patient sample, Lane 4 – positive control (classical bcr1 isoform, 381 bp), Lane 5 – water control; Line 6 – 100 bp DNA marker; Lanes 7 – 10 represent PCR amplification using PML-A2/RAR-α-B primers: Lane 7 – negative control, Lane 8 – patient sample, Lane 9 – positive control (classical bcr3 isoform, 376 bp), Lane 10 – water control.

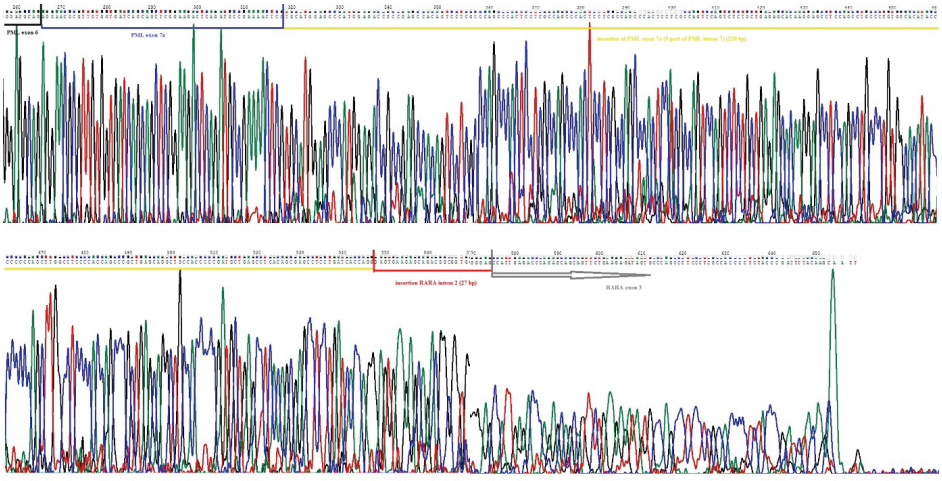

Fig. 2 – Sequencing analyses of the 1st round polymerase chain reaction (PCR) product showing atypical promyelocytic leukemia (PML)/retinoic acid receptor (RAR)-α fusion transcript (reverse complement).

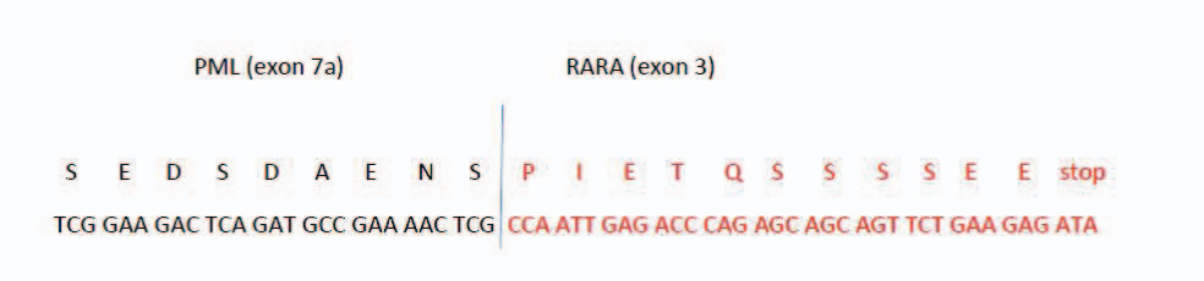

Fig. 3 – cDNA and amino-acid sequence of the promyelocytic leukemia (PML)/retinoic acid receptor (RAR)-α junction between PML exon 7a and RAR-α exon 3 present in our patient, represented as an inferior malignant clone (Figure 1, Lane 3).

The weaker, shorter band, in addition of complete PML exon 6, contained exon 7a (53 bp), that was directly fused to RAR-α exon 3 forming a band of 434 bp. This fusion transcript was not in-frame, since the direct joining of the PML 7a exon to the RAR-α exon 3 generates stop codon in the exon 3 RAR-α gen, precisely at the codon coding for the 12th amino acid of the RAR-α exon 3 (Figure 3).

Discussion

Herein we present a very rare case of the cytogenetically cryptic, FISH-negative, but RT-PCR PML/RAR-α positive APL patient. To our knowledge there has been several cytogenetically cryptic, FISH-negative cases described in the literature [19, 20]. Still, none of these cases refers to such an atypical PML/RAR-α transcript found in our patient.

The reasons for not detecting t(15;17) during routine cytogenetic analyses can be numerous ranging from poor morphology, complex karyotype, but most likely the reason is the presence of submicroscopic insertions leading to cryptic PML/RAR-α fusions [21, 22]. However, the majority of those, cytogenetically cryptic cases, can be detected using FISH (dual-colour or dual fusion probes) analyses. The reports in which the FISH analyses for the PML/RAR-α fusions were negative, while RT-PCR analyses were positive are very rare [19, 21, 23, 24]. These findings can be explained as a consequence of very small insertions, too small for the probe to hybridize to, or the signal that probe produces is too faint to be detected. The difference between all those previously published cases and our case is that in all of them RT-PCR analyses reported the bcr1 or bcr3 isoform. Only one study reported the bcr2 case, but none of them was the case of rare PML/RAR-α transcript with the breakpoint in PML gene after the exon 6 [20].

In our patient we detected the presence of two PML/RAR-α transcripts both with breakpoint after PML exon 7a. The dominant transcript contained entire PML exon 6, exon 7a, 5` part of PML intron 7 (also called PML exon 7b), followed by an insertion of RAR-α intron 2 speeding out through RAR-α exon 3. In addition to this in-frame fusion transcript, we detected a minor PML/RAR-α transcript which differed from the dominant one in that it did not contain neither 5` part of *PML* intron 7, nor the RAR-α intron 2 insertion. Its PML exon 7a was directly fused to RAR-α exon 3, forming an out-of-frame transcript.

In the literature there are only 3 studies describing cases similar to the one here reported (Table 1). Chillon et al. [14] were the first to report two APL patients in which a breakpoint position was outside the coding sequence of exon 7a. In both of the analysed patients the same out-of-frame transcript (PML exon 7a/RAR-α exon 3) as in our patient was detected. One of the patients described had a PML/RAR-α fusion transcript similar to the dominant transcript found in our patient, but without insertion of the part of RAR-α intron 2. This transcript was like in our case in-frame.

In the study by the Barragán et al. [13] in one of the two reported cases, sequencing analyses of the PML/RAR-α fusion transcript showed the presence of the 35 bp insertion originating from RAR-α intron 2, as in our patient. It has been known that the breakpoint in RAR-α gene in t(15;17) is almost always located in the RAR-α intron 2, but there are some cases in which the part of the RAR-α intron 2 is inserted into PML/RAR-α fusion transcript. There are examples of RAR-α intron 2 insertions found in different bcr2 isoforms [25]. Generally speaking, most likely to be inserted are the sequences from RAR-α intron 2 sites that are most prone to breakage. These sites are numerous and spread throughout this 17 kb large intron. Insertion of genomic DNA in the case of bcr2 transcript has been described by others to [4, 7, 13, 14, 16, 26]. There are speculation about the necessity for that part of the DNA sequence in order to ensure an in-frame sequence of the bcr2 transcript [25]. Sometimes the breakpoint in RAR-α gene can be detected inside exon 3, like in the APL patient presented by Park et al. [27]. Namely, one of the PML/RAR-α transcripts detected in the APL patient contained full PML exon 7a, insertion of PML intron and the deletion of first 46 bp from the RAR-α exon 3.

In all of the previously mentioned studies, including our own, a total of 5 patients whose break point in the PML/RAR-α transcript is after exon 7a of PML gene were described. That makes our finding even more significant. The question that remains is: Is the presence of atypical PML/RAR-α transcript causing different response to ATRA therapy? There are reports that patients with different PML/RAR-α fusion transcripts exhibit different sensitivity to ATRA. Significantly worse prognosis of the bcr3 positive patients compared to bcr1 patients have been described [28, 29]. Also, it was shown that patients with bcr2 PML/RAR-α transcript had reduced sensitivity to ATRA [30].

Table 1

Summary of atypical PML/RAR-α fusion transcripts with an insertion of PML exon 7a [13, 14, 27]

Case	Sex	Age, years	Karyo-type	Sequencing analyses of the RT-PCR product (from PML exon 6 to RARα exon 3)	Treatment	Survival	Reference
				3 types of transcript:			
1	M	28	t(15;17)	PML exon6, exon 7a, 5`part of exon 7b / RARα exon 3 (in-frame) PML exon6, exon 7a /RARα exon 3 (out-of-frame) PML exon 6 deletion, exon 7a / RARα exon 3 (out-of-frame)	No	Dead at day 2 (intracerebral hemorrhage)	[14]
2	M	28	t(15;17)	PML exon6, exon 7a / RARα exon 3 (out-of-frame)	ATRA + chemotherapy	Dead at day 13 (multiorgan failure)	[14]
3	M	50	NA*	PML exon6, exon 7a /RARα exon 3 (out-of-frame)	LPA-99 protocol	Dead at day 16 (multiorgan failure)	[13]
4	M	38	t(15;17)	PML exon6, exon 7a, insertion of intron 7/ RARα exon 3 deletion (46bp) (in-frame)	ATRA + anthacycline	Dead at day 14 (pulmonary hemorrhage)	[27]
				2 types of transcript:			
5	F	62	Cryptic, FISH-negative	PML exon6, exon 7a, 5`part of exon 7b / RARα intron 2 insertion (27bp), RARα exon 3 (in-frame) PML exon6, exon 7a / RARα exon 3 (out-of-frame)	ATRA + pred-nisone	Dead at day 1 (intracerebral hemorrhage)	Present case

***NA – not available; RT-PCR – reverse transcriptase polymerase chain reaction; PML – promyelocytic leukemia; ATRA – all-trans retinoid acid; RAR – retinoid acid receptor; FISH – fluorescence *in situ* hybridization.**

This was later confirmed by *in vitro* studies [16]. In our study APL patent died too soon for the conclusion about the sensitivity to ATRA to be made. The same outcome was detected in patients reported by other studies [13, 14, 27]. Sadly, the number of reported patients (five) is too small to draw any kind of conclusion regarding prognostic relevance of reported atypical PML/RAR-α transcript.

Conclusion

Our results are an important addition to the limited number of reports describing the existence of atypical PML/RAR-α fusion transcripts in APL patients. Namely, this is only the fifth case of the APL patient with PML exon 7a incorporated in the PML/RAR-α transcript, associated with aggressive course of the disease. In order to clarify the biological significance and prognostic value of atypical PML/RAR-α fusion transcripts like the one reported in our study, it is important to address to every such case and apply an extended analysis including detailed characterization and sequencing of the fusion transcript.

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Ethics

This study adhered to the tenets of the Declaration of Helsinki. We obtained written informed consent from the patient, and also the study was approved by the Ethical Committee of the Clinic of Hematology, Clinical Center of Serbia.

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

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Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure – **Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi – **Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhođane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

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

Primeri referenci:

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Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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