YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

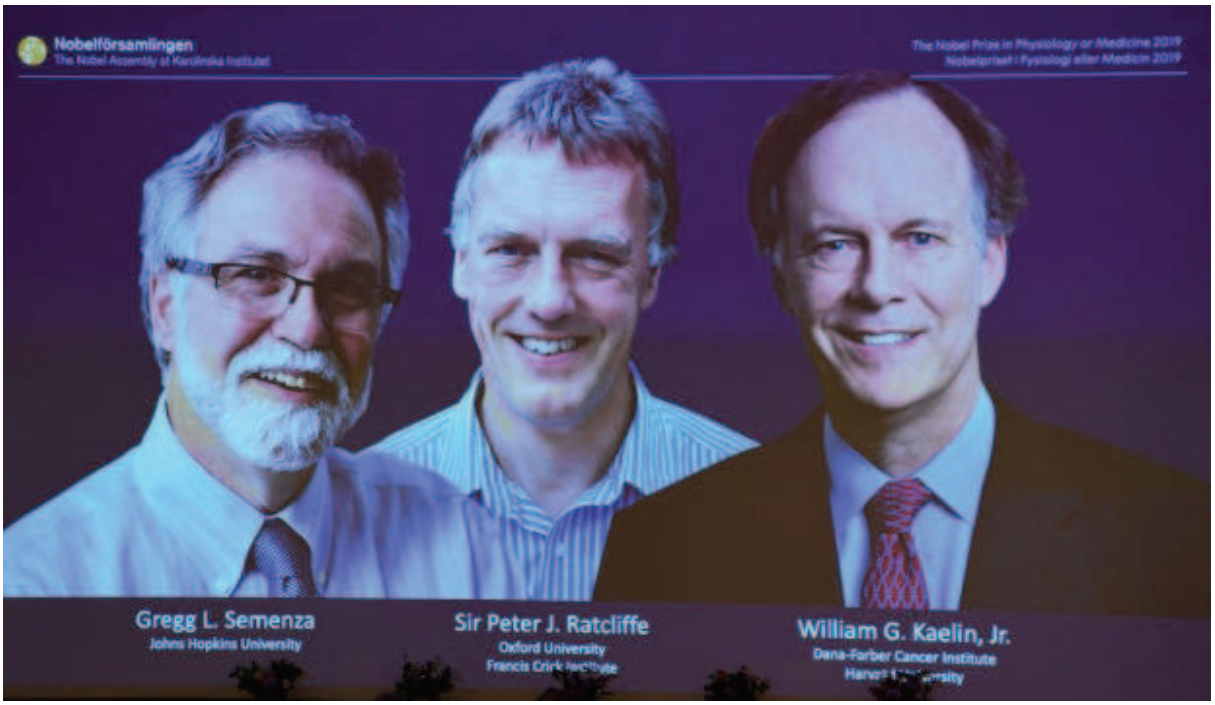
Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2019; October Vol. 76 (No. 10): p. 975–1098.

Vojnosanitetski Pregled 2019 October Vol. 76 (No. 10): p. 975–1098.



vol. 76, br. 10, 2019

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine

Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Univerzitet odbrane, MO Republike Srbije

ISSN 0042-8450
eISSN 2406-0720
Open Access
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Adresa redakcije: Univerzitet odbrane, Institut za naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija.
Informacije o pretplati: Tel.: +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu“ indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa Vojna štamparija, Beograd, Resavska 40b

vol. 76, No. 10, 2019

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of *Vojno-sanitetski glasnik* which was published between 1930 and 1941

PUBLISHER
University of Defence, Ministry of Defence of the Republic of Serbia, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Editorial Office: University of Defence, Institute for Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia.
E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

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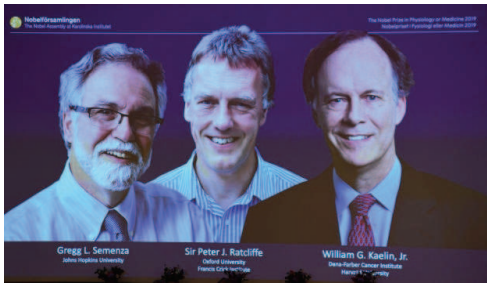

The winners of the 2019 Nobel Prize in physiology or medicine are (from left to right): Gregg L. Semenza (Johns Hopkins University, USA), Sir Peter J. Ratcliffe (the Francis Crick Institute and Oxford University, Great Britain) and William G. Kaelin (Harvard University, USA). All three scientists worked independently over a period of more than two decades to establish how cells can sense and adapt to changing oxygen availability. They identified molecular machinery that regulates the activity of genes in response to varying oxygen levels that has paved the way to the new therapeutic strategies to fight anemia, cancer and other diseases

S leva na desno, dobitnici Nobelove nagrade za medicinu u 2019. godini: Gregg L. Semenza (Johns Hopkins University, USA), Sir Peter J. Ratcliffe the Francis Crick Institute and Oxford University, Great Britain) i William G. Kaelin (Harvard University, USA). Sva tri naučnika, tokom više od 20 godina, nezavisno su radili na otkrivanju načina kako ćelije mogu osetiti i kako se mogu adaptirati na promene u dostupnosti kiseonika. Oni su identifikovali molekularnu mašineriju koja reguliše aktivnost gena u zavisnosti od varijacija u nivou kiseonika što je utrlo put novim terapijskim strategijama u borbi protiv anemije, kancera i drugih bolesti.

ORIGINAL ARTICLE

UDC: 616.379-008.64:[616.127-005.8-08-036
https://doi.org/10.2298/VSP170905175M

Prognostic significance of inflammatory biomarkers in diabetic and non-diabetic patients with STEMI, treated with primary percutaneous coronary intervention

Prognostički značaj biomarkera inflamacije kod bolesnika sa i bez dijabetesa koji su lečeni primarnom perkutanom koronarnom intervencijom zbog akutnog infarkta miokarda sa elevacijom ST segmenta

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Abstract

Background/Aim. Although the prognostic significance of inflammatory biomarkers, C-reactive protein (CRP) and fibrinogen, in the patients with the ST-segment elevation myocardial infarction (STEMI) is already known, the specific difference between such patients according to diabetic status remains unknown. **Methods.** The study was conducted in a single tertiary center. The values of CRP and fibrinogen were measured during the first 48 h in consecutive patients with first STEMI treated with primary percutaneous coronary intervention (pPCI). The patients were divided into two groups: with diabetes and without diabetes. The aim of this study was to determine a prognostic significance of maximal values of these two inflammatory biomarkers for in-hospital and six-month mortality in these two groups. **Results.** Among 475 patients, 126 (26.5%) were with diabetes and 349 (73.5%) were without diabetes. The patients with diabetes had significantly higher median values of CRP and fibrinogen compared to the nondiabetic patients [29.6 (10.4–91.8) mg/L *vs* 22.4 (9.79–49.2) mg/L, $p = 0.046$ and 4.7 (3.6–6.3) g/L *vs* 4.3 (3.6–5.4) g/L, $p = 0.026$, respectively]. However, the multivariate survival analysis using the Cox regression model showed that in the nondiabetic STEMI patients CRP and fibrinogen had significant prognostic value for in-hospital mortality [hazard ratio (HR) = 1.013, 95% confidence interval (CI) (1.004–1.022), $p = 0.004$; HR = 1.529 (1.023–2.287), $p = 0.039$, respectively]. Regarding six-month mortality, no significant difference was achieved. Overall survival was the lowest in the fourth quartile of CRP in the patients without diabetes. **Conclusion.** The higher values of CRP are the significant independent predictor of in-hospital and overall mortality in the STEMI patients without diabetes treated with primary PCI. Fibrinogen can also be used as an additional prognostic inflammatory biomarker for in-hospital mortality in the non-diabetics with STEMI.

Key words:
biomarkers; c-reactive protein; diabetes mellitus; fibrinogen; mortality; percutaneous coronary intervention; prognosis; st elevation myocardial infarction.

Apstrakt

Uvod/Cilj. Iako je poznat prognostički značaj inflamatornih biomarkera kao što su C-reaktivni protein (CRP) i fibrinogen, kod bolesnika sa akutnim infarktom miokarda sa elevacijom ST-segmenta (STEMI), postojanje razlike u zavisnosti od prisustva ili odsustva dijabetesa nije poznato. **Metode**. Istraživanje je sprovedeno u medicinskom centru tercijernog nivoa. Kod bolesnika sa STEMI, lečenih primarnom perkutanom koronarnom intervencijom (pPKI) merene su vrednosti CRP-a i fibrinogena, u toku prvih 48h od prijema. Bolesnici su bili podeljeni u dve grupe: grupu sa dijabetesom i grupu bez dijabetesa. Cilj rada bio je da se utvrdi prognostički značaj maksimalnih vrednosti ta dva biomarkera zapaljenja za nastanak intrahospitalne i šestomesečne smrtnosti u svakoj od grupa. **Rezultati**. Među 475 bolesnika, 126 (26,5%) je imalo dijabetes, a 349 (73,5%) nije imalo dijabetes. Bolesnici sa dijabetesom su imali značajno veću vrednost medijane CRP-a i fibrinogena u poređenju sa bolesnicima bez dijabetesa [29,6 (10,4–91,8) *vs* 22,4 (9,79–49,2) mg/L, $p = 0,046$ i 4,7 (3,6–6,3) g/L *vs* 4,3 (3,6–5,4) g/L, $p = 0,026$]. Međutim, korišćenjem Cox regresione multivarijant-

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ne analiza smrtnosti modela pokazano je da su kod bolesnika bez dijabetesa CRP i fibrinogen imali značajnu prognostičku vrednost za nastajanje intrahospitalne smrtnosti [*hazard ratio* – stopa rizika (HR) = 1,013 95% interval poverenja (CI) (1,004–1,022), $p = 0.004$ i HR = 1,529, 1,023–2,287 95%CI, $p = 0{,}039$]. Kada je u pitanju šestomesečna smrtnost, nije pronađena statistički značajna razlika. Ukupno preživljavanje je bilo najniže u četvrtom kvartilu CRP-a u grupi bolesnika bez dijabetesa. **Zaključak**. Visoke vrednosti CRP-a su nezavisan prediktor intrahospitalne i ukupne šestomesečne smrtnosti kod bolesnika sa STEMI koji nemaju dijabetes lečenih pPKI. Fibrinogen se takođe može koristiti kao prognostički marker za intrahospitalnu smrtnost kod nedijabetičara sa STEMI.

Ključne reči:
biomarkeri; c-reaktivni protein; dijabetes melitus; fibrinogen; mortalitet; perkutana koronarna intervencija; prognoza; infarkt miokarda sa st elevacijom.

Introduction

Inflammation is the inevitable companion of acute myocardial infarction and plays a key role in wound healing and scar formation [1]. Different patterns of inflammatory response are detected between myocardial infarction with the ST-segment elevation (STEMI) and without STEMI (NSTEMI) [2]. C-reactive protein (CRP) and fibrinogen, the acute phase reactants, are commonly used in everyday clinical praxis. The role of CRP and fibrinogen as predictors of heart failure development and mortality after acute myocardial infarction are already investigated [3–8]. Diabetes is well-known risk factor for cardiovascular disease, but it is also associated with the increased inflammation. The increased inflammatory biomarkers are even suspected to cause diabetes mellitus type 2 and its complications [9]. The patients with STEMI are often treated with primary percentanous coronary intervention (pPCI) with a stent implantation. Even this intervention itself is associated with an increased inflammatory response [10, 11]. However, it is still unknown if there are any differences in the inflammatory response between the patients with and without diabetes and with acute myocardial infarction treated with pPCI. In addition, it is still unclear whether the prognostic value of some inflammatory biomarkers in these two subgroups of patients observed separately is different.

We measured CRP and fibrinogen during the first two days of hospitalization in consecutive STEMI patients treated with pPCI, and depending on their diabetic status, we evaluated the prognostic value of these two biomarkers for early (in-hospital) and late (six-month) mortality.

Methods

This is a retrospective and partly prospective cohort study performed in the Clinic for Emergency Internal Medicine at the Military Medical Academy in Belgrade, Republic of Serbia in the period from 2002 till 2016. Only the patients admitted due to the first, ever diagnosed STEMI who underwent pPCI and with all available data were included in the study. STEMI was diagnosed according to electrocardiogram (ECG) recorded as pre-hospital or at admission. The ST-segment elevation in two adjacent leads by ≥ 1 mm in leads I–III, aVF, aVL, V4–V6 and ≥ 2 mm in leads V1–V3, were considered as significant for the diagnosis [12]. All patients included in the study underwent pPCI in the time frame no longer than 12 h from the self-reported chest pain onset. Before the PCI execution, all patients received loading doses of aspirin (300 mg) and clopidogrel (600 mg), or ticagrelor (180 mg). The venous blood samples for determination of CRP and fibrinogen were collected once a day during the first 48 h from the admission starting in the morning after the pPCI procedure. The maximal values of CRP and fibrinogen were then evaluated for the prediction of in-hospital and six-month mortality. For overall mortality, the quartiles of maximal values of these inflammatory biomarkers were compared for the frequencies of event. In-hospital mortality was considered as death during the hospital stay starting from the first day from the admission after successful pPCI. Six-month mortality was considered as any cause of death starting from day 30 until day 180 from the admission.

Statistics

For the data analysis, the maximal values of CRP and fibrinogen were expressed as the median with interquartile range (IQR). The categorical data were expressed as numbers (percentages). The Fisher's exact test for the qualitative variables and the Mann-Whitney *U* test for the quantitative variables were used to test a difference between the groups. For the multivariate survival analysis, the Cox regression model was used and overall survival was depicted by the Kaplan-Meier curve using the log-rank test to compare the survival distributions among the quartile groups. All statistics were performed using the SPSS for Windows, version 20.0. *P* value < 0.05 was considered to be significant.

Results

Overall 475 consecutive patients with the first STEMI treated with pPCI were included in the study. Among them, 126 (26.5%) were diagnosed with diabetes type 2 and 349 (73.5%) did not have diabetes. The patients with diabetes were older, more often female and more often hypertensive compared to nondiabetics. They were also presented more often with the acute heart failure (Killip class > 1) and with longer time that passed from the pain onset to reperfusion (pPCI). In opposite, the nondiabetics were more often smokers than the diabetics. Other basic characteristics of the patients were presented in Table 1.

In the diabetic group, in-hospital mortality was 11 (8.7%) patients and six-month mortality was 9 (7.1%) pa-

tients. In the nondiabetic group, in-hospital mortality was 18 (5.2%) patients and six-month mortality was 25 (7.2%) patients. The patients with diabetes had the significantly higher median values of CRP and fibrinogen compared to the nondiabetic patients [29.6 (10.4–91.8) mg/L *vs* 22.4 (9.79–49.2) mg/L, $p = 0.046$ and 4.7 (3.6–6.3) g/L *vs* 4.3 (3.6–5.4) g/L, $p = 0.026$, respectively]. Adjusted hazard ratios (HR) for the presence of two outcomes considering the maximal values of CRP and fibrinogen observed separately for the patients with diabetes and without diabetes are shown in Table 2.

When using the stepwise regression model, CRP emerged as the best independent predictor of in-hospital mortality, followed by the years of age in the patients without diabetes. When fibrinogen was included in the stepwise regression model, years of age had advantage as an independent predictor of in-hospital mortality, followed by fibrinogen in the patients without diabetes.

In the patients without diabetes, overall mortality during the first six months was 12.3% and 15.9% in the patients with diabetes. A significantly lower overall survival was in the fourth quartile of CRP compared to other three quartiles among the patients without diabetes. No significant difference in overall survival between the quartiles of CRP was found among the patients with diabetes (Figure 1). The maximal values of fibrinogen did not show a significance in term of overall six-month mortality in the patients with and without diabetes (Figure 2).

Discussion

This study showed that among the STEMI patients treated with p/PCI, those with diabetes type 2 had the significantly higher values of CRP and fibrinogen compared to the patients without diabetes. However, a prognostic significance of these inflammatory biomarkers for the prediction of mortality was completely different. A higher value of CRP was a significant predictor of in-hospital mortality in the nondiabetics, but not in the diabetics. In addition, in the patients without diabetes, the higher values of fibrinogen had the additional prognostic implication on in-hospital mortality. The higher values of CRP and fibrinogen were not associated with higher six-month mortality after the patients were discharged from the clinic.

Table 1

Basic characteristics at the admission of the patients stratified by presence or absence of diabetes

Characteristics	Diabetics 126 (26.5%)	Non-diabetics 349 (73.5%)	*p*
Age, (years), mean ± SD	64.2 ± 12.2	61.6 ± 12.2	0.038
Female, n (%)	45 (35.7)	88 (25.2)	0.037
Smokers, n (%)	52 (41.2)	197 (56.4)	0.003
Hypertension, n (%)	97 (76.9)	229 (65.6)	0.034
Hypercholesterolemia > 5 mmol/L, n (%)	72 (57.1)	223 (63.9)	0.216
Modified Selvester ECG score > 15%, n (%)	53 (42.1)	126 (36.1)	0.569
Q-wave on the admission ECG, n (%)	53 (42.1)	131 (37.5)	0.448
Killip class > 1, n (%)	33 (26.1)	51 (14.6)	0.006
Time to reperfusion in hours, mediana (25th–75th IQR)	5.0 (3.0–8.0)	3.5 (2.0–6.0)	0.005
Multivessel coronary disease, n (%)	90 (70.7)	215 (61.8)	0.081
Infarction related artery, n (%)			
left main	2 (1.6)	4 (1.1)	0.660
LAD	45 (35.7)	142 (40.7)	0.340
CXa	18 (14.2)	46 (13.2)	0.764
RCA	56 (44.4)	140 (40.1)	0.463
TIMI flow at the end of pPCI, n (%)			
TIMI < 3, n (%)	18 (14.2)	62 (17.8)	0.407
Rezolution of ST-segment after pPCI < 50%, n (%)	49 (38.9)	103 (29.5)	0.068

LAD – left anterior descending artery; CXa – circumflex artery; RCA – right coronary artery; pPCI – primary percutaneous coronary intervention; TIMI – thrombolysis in myocardial infarction; IQR – interquartile range.

Table 2

The multivariate survival analysis using the Cox regression model in the STEMI patients with diabetes

Parameter	HR (95% CI); *p*	
	CRP	Fibrinogen
In-hospital mortality		
with diabetes	1.015 (0.994–1.037); 0.173	2.169 (0.879–5.351); 0.093
without diabetes	1.013 (1.004–1.022); 0.004	1.529 (1.023–2.287); 0.039
Six-month mortality		
with diabetes	1.002 (0.984–1.019); 0.856	2.506 (0.897–6.993); 0.079
without diabetes	1.005 (0.997–1.013); 0.191	1.241 (0.826–1.866); 0.298

***Adjusted for age, gender, smokers, hypertension, Killip class and time to reperfusion.**

CRP – C-reactive protein; HR – hazard ratio; CI – confidence interval; STEMI – ST-segment elevation myocardial infarction.

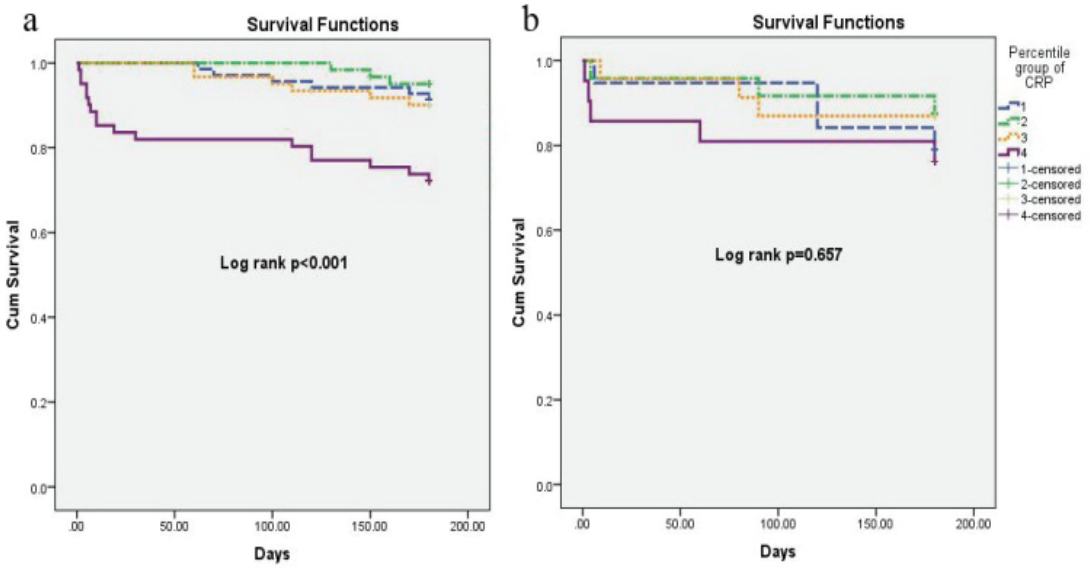

Fig. 1 – a) Kaplan-Meier curves depict overall six-month survival among: a) the nondiabetic patients and b) the diabetics patients according to the C-reactive protein (CRP) quartiles.

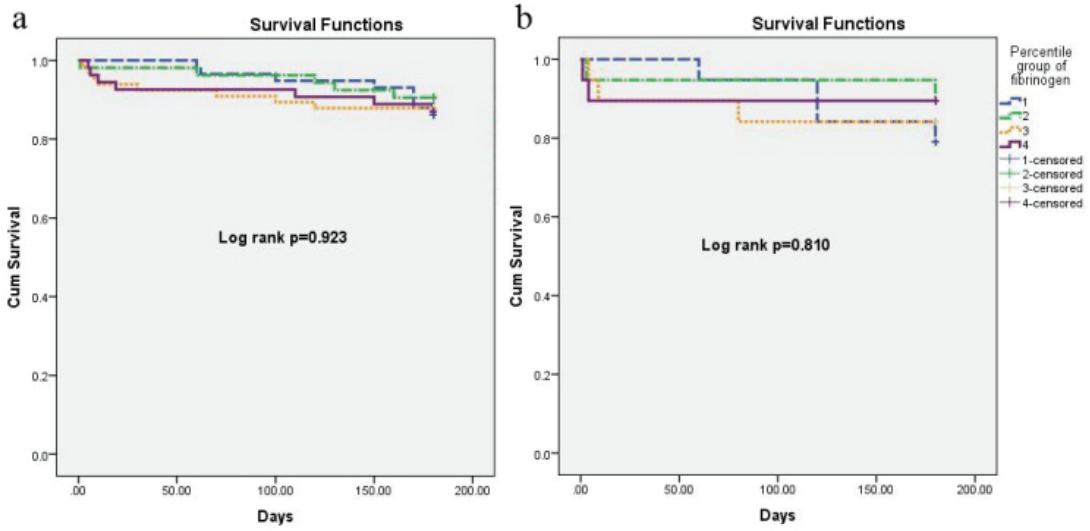

Fig. 2 – Kaplan-Meier curves depict overall six-month survival among: a) the nondiabetic (patients) and b) the diabetics patients according to the fibrinogen quartiles.

Even though the chronic inflammation increases atherosclerosis and correlates with extended cardiovascular disease, it seems that the STEMI patients without diabetes are more prone to the worst early outcome if these two inflammatory biomarkers are highly elevated. Several studies already proved a high correlation between the high values of CRP and fibrinogen at the hospital addmission in the STEMI patients and a high risk of early mortality, but neither addressed the presence, or absence of diabetes mellitus as an issue that can influence different prognostic implications of CRP or fibrinogen on mortality [4, 13–15]. Other inflammatory biomarkers are also linked to mortality in the patients with acute myocardial infarction, although their measurement is more expensive and not widely available in clinical practice [16–18]. In our study, the STEMI patients without diabetes who were in the fourth quartile value of CRP had a higher incidence of overall mortality, and fibrinogen did not have prognostic implications on six-month overall mortality in both groups. The influence of other risk factors on CRP values such as smoking cigarettes, presence of arterial hypertension, or even female gender are reported in some papers [18–20]. Prolonged ischemic time has also influence on the increased CRP levels [21]. However, in the multivariate survival analyses, when putting all these risk factors into one model using the Cox regression, CRP still emerges as the best independent predictor of early mortality in the patients without diabetes. The multivariable regression analysis also recognized fibrinogen as a significant predictor of in-hospital mortality in the patients without diabetes. However, there was no significance for six-month mortality.

The reason for this difference in a prognostic significance of inflammatory biomarkers, in particular CRP, between the STEMI patients with and without diabetes is still unknown. One of the possible answers might be that the acute glucose fluctuation in the nondiabetic STEMI patients triggers more oxidative stress compared with sustained chronic hyperglycemia in the patients with diabetes type 2 [22]. It is also known that acute hyperglycemia in STEMI is an independent risk factor for adverse events and that can even potentiate stress-induced apoptosis [19–21].

The importance of laboratory measurement of inflammatory biomarkers for the assessment of mortality risk in the STEMI patients after percutaneous angioplasty could help in individualizing the treatment and follow-up schedules for these patients.

Study limitations

This study included relatively low number of STEMI patients with diabetes leading to the low number of events in this group of patients. The data for six-month mortality was obtained mostly by telephone contact with the patients family and did not include the cause of death, although injuries and other similar accidents were excluded. Our laboratory also did not use thehigh sensitive CRP (hs-CRP) tests which were unavailable.

Conclusion

Although the STEMI patients with diabetes, treated with primary PCI, had the higher values of CRP and fibrinogen on hospital admission, comparing to patients without diabetes, these inflammatory biomarkers, in particular CRP, were the significant predictors of in-hospital mortality only in the patients without diabetes. Regarding overall six-month mortality in the STEMI patients without diabetes treated with primary PCI, those with CRP values in fourth quartile had a significantly higher incidence of death comparing to those with CRP values in lower quartiles.

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Received on September 05, 2017.
Revised on November 06, 2017.
Accepted on November 08, 2017.
Online First November, 2017.

ORIGINAL ARTICLE

UDC:616.314-007:616.89-008.454
https://doi.org/10.2298/VSP171011182C

Association of health determinants and depressive symptoms with tooth loss in the Serbian adult population: a cross-sectional study

Povezanost determinanti zdravlja i simptoma depresije sa nedostatkom zuba kod odraslog stanovništva Srbije: studija preseka

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Abstract

Background/Aim. Oral diseases appear to be the major risk factors for tooth loss, but social factors and other determinants could play an important role as well. The aim of this study was to determine whether the possible independent sociodemographic risk factors and depressive disorders could contribute to the tooth loss in the adult population of Serbia. **Methods.** This cross-sectional study analysed the 2013 National Health Survey results for the population of Serbia (without the data for Kosovo and Metohia population). The sample was selected to provide the statistically reliable estimates at the national level. Study included 13,519 adults of 20 years of age, or above. The mean age of participants was 49.9 years including 6,998 (51.8%) females and 6,518 (48.2%) males. The number of missing teeth was dependent variable while the independent variables were: gender, age, marital status, education, employment status, Wealth Index, smoking status, body mass index (BMI), milk and milk products intake, fruit intake excluding juices, vegetable and salad intake (excluding potato), and depression. To measure the current depression, the standardized and validated the Patient Health Questionnaire (PHQ)-8 score was used. Edentulism (complete or partial) was defined as a loss of natural teeth. In order to determine the predictors of tooth loss, the bivariate and multivariate logistic regression models were implemented for all types of tooth loss. **Results.** The significant differences were observed among the categories of edentulism in relation to independent variables except vegetable and salad intake. The prevalence of all missing teeth was highest in the females, the respondents with a low level of education, inactive, underweight (BMI < 18.5) and respondents with moderately severe depressive episodes (PHQ-8 score 15 to 19). In the multivariate model, we found that the demographic factors (age, living with a partner), socioeconomic factors (primary and secondary education, Wealth Index), smoking, BMI, the PHQ-8 score and the depressive symptoms were significantly associated with partial tooth loss. **Conclusion.** Study demonstrated that sociodemographic and lifestyle factors as well as the symptoms of depresssion are also important factors related to the tooth loss. This study can help to advance the need for health programs focusing on education, smoking cessation, dietary habits as well as regular visits to the dentist.

Key words:
adult; depression; jaw, edentulous; risk factors; socioeconomic factors; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Bolesti usta i zuba su glavni faktor rizika od gubitka zuba, ali i socijalni faktori i druge determinante zdravlja takođe mogu imati važnu ulogu. Cilj istraživanja je bio da se utvrdi da li potencijalni nezavisni faktori, kao što su sociodemografski faktori, stil života i depresivni poremećaji, mogu doprineti gubitku zuba odraslog stanovništva Srbije. **Metode.** Analizirani su podaci dobijeni u okviru Nacionalnog istraživanja zdravlja stanovništva Srbije koje je sprovedeno 2013. godine kao studija preseka na reprezentativnom uzorku odraslog stanovništva Srbije (bez podataka o stanovništvu Kosova i Metohije). Uzorak je izabran da obezbedi statistički pouzdane procene na nacionalnom nivou. Studija je obuhvatila 13 519 odraslih osoba starosti 20 godina i više. Prosečna starost ispitanika, uključujući 6 998 (51,7%) žena i 6 517 (48,2%) muškaraca bila je 49,9 godina. Kao zavisna varijabla analiziran je broj zuba koji su nedostajali ispitanicima, dok su nezavisne varijable bile: pol, starost, bračno stanje, obrazovanje, radni status, Indeks blagostanja, pušački

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status, indeks telesne mase (BMI), unos mleka i mlečnih proizvoda, voća (isključujući voćne sokove), povrća i salata (isključujući krompir) i depresija. Za utvrđivanje prisustva depresivne epizode korišćen je standardizovan Upitnik o zdravlju pacijenata – *Patient Health Questionnaire* (PHQ)-8. Gubitak prirodnih zuba definisan je kao bezubost (potpuna ili delimična). Za određivanje prediktora gubitka zuba korišćeni su modeli bivarijantne i multivarijantne logističke regresije za sve kategorije gubitka zuba. **Rezultati.** Nađena je statistički značajna razlika između kategorija bezubosti u odnosu na sve nezavisne promenljive izuzev unosa povrća i salata. Prevalencija totalne bezubosti bila je najveća kod žena, niskoobrazovanog i neaktivnog stanovništva, pothranjenih (BMI < 18,5 kg/m^2) i ispitanika sa umereno teškim depresivnim epizodama (PHQ-8 skor od 15 do 19). Multivarijantnim modelom je utvrđeno da su demografski faktori (životno doba, život sa partnerom), socijalnoekonomski faktori (niže i srednje obrazovanje, Indeks blagostanja), pušenje, BMI, PHQ-8 skor i prisustvo depresivnih simptoma bili statistički značajno povezani sa delimičnim gubitkom zuba. **Zaključak.** Ova studija ukazuje na to da sociodemografski faktori, stil života i depresivni simptomi predstavljaju faktore koji mogu biti povezani sa gubitkom zuba. Studija ukazuje na neophodnost donošenja programa koji su usmereni na obrazovanje, prestanak pušenja cigareta, navike u ishrani, kao i redovne posete stomatologu.

Ključne reči:
odrasle osobe; depresija; bezubost; faktori rizika; socijalno-ekonomski faktori; ankete i upitnici.

Introduction

Over the last few years, an increasing number of epidemiological studies were carried out around the world to determine the state of oral health [1, 2]. Complete or partial tooth loss has been recognized as the final outcome of the mouth and teeth diseases, health behavior, preferences, or professional intervention [3]. Burt et al. [4] concluded that the complete tooth loss is not just a disease-related extension of partial tooth loss, but rather a different phenomenon with the social and attitudinal factors playing a prominent part. It is well- known that the social factors and other determinants influence the health outcomes [5]. The results of studies of health inequalities showed that inequalities were most often related to education, occupational class, income and demographic characteristics such as gender and age [6, 7]. Oral diseases appear to be the major risk factors for partial tooth loss, suggesting that the sociodemographic and lifestyle factors and depressive disorders play an important role [8, 9]. Furthermore, the behavioral factors such as smoking, unhealthy dietary patterns and inadequate consumption of milk, fruit and vegetable are believed to contribute to tooth loss as well [3, 10, 11]. On the other hand, oral status may cause suffering and has a great impact on the general quality of life (dysfunction of the oral cavity and facial aesthetics, absenteeism, social isolation, etc.) [12].

Nowadays, the depressive disorders are one of the leading health challenges of the 21st century due to its high prevalence and constant growth [13]. Depressive symptoms have been correlated to the poorer self-assessed oral health and oral-health-related quality of life [14, 15]. It was estimated that one in five dental patients may have a depressive disorder [16].

Since 2005, there were significant changes in legal regulations, concerning that the Serbian adult population 18+ costs of dental services (with exception of pregnant women, new mothers and those over 65 years of age) influenced a lot the provision and use of dental health care. According to the National Health Survey of 2013, only 26.9% of respondents had their own dentist in the state-owned institution and 31% in private practice [17].

The aim of this study was to determine whether the possible independent sociodemographic, risk factors and depressive disorders could contribute to tooth loss in the adult population of Serbia.

Methods

This study represents an analysis of the 2013 National Health Survey for the population of Serbia (without the data on Kosovo and Metohia population), which was carried out by the Ministry of Health of the Republic of Serbia. The study protocol was approved by the Ethics Review Board of the Institute of Public Health of Serbia. A stratified two-stage representative sample of the Serbian population was used for this study. The sample was selected to provide the statistically reliable estimates at the national level and at the levels of 4 geographical regions of Serbia (Province of Vojvodina, Belgrade, Central and West Serbia, South and East Serbia).

Out of 10,089 households from all registered households in 2011in the Republic of Serbia, 6,500 households were randomly selected for the sample (3,960 urban and 2,540 rural) and interviewed during October-December 2013. The interviews and measurements were carried out in each household by teams consisting of two trained interviewers and a healthcare worker. The informed written consent was obtained from all respondents. The household response rate was 64.4%. Of the total of 16,474 registered members of the household aged 15 years and above, 14,623 were interviewed, giving a response rate of 88.9%. Of this number of people who agreed to be interviewed, 13,756 of them accepted to fill a self-administered questionnaire (response rate 94.1%). All measurements were performed on 13,908 respondents aged 15 years and above (response rate 95.1%), and a partial measurement for another 534 respondents [17]. For the purpose of this study, we analyzed the data on participants aged 20 years or above, the total of whom was 13,519 adults. The mean age of participants was 49.9 years [including 6,998 (51.7%) females and 6,517 (48.3%) males].

Edentulism (complete or partial loss of natural teeth) is a debilitating and irreversible condition and is described as the "final marker of disease burden for oral health" [18]. The number of missing teeth was dependent variable, and it was assessed within 5 category items (no missing teeth, 1–5 missing teeth, 6–10 missing teeth, 10 or more missing teeth, and all teeth missing). In addition, a series of demographic and socioeconomic variables that could be related to edentulism were included: gender, age (20–34, 35–44, 45–54, 55–64 and 65 years and above), type of settlement (urban or rural), marital status (living with a partner or without partner), education (primary, secondary, or high), employment status (employed, unemployed, or inactive) and the Wealth Index (Demographic and Health Survey Wealth Index) according to which the households and respondents were classified into five socioeconomic categories (poorest, poorer, middle class, rich or the richest class). The Wealth Index was calculated using the data on a household ownership of selected assets, such as number of bedrooms per household member, materials used for housing construction, types of water access and sanitation facilities, possession of color TV, mobile phone, refrigerator, computer, washing machine, dishwasher, air conditioning, central heating, car and access to the internet [19]. The lifestyle variables included smoking status (never smoked, past smoker or smoker), body mass index (BMI; the weight and height were measured according to a defined protocol and used to calculate the BMI as weight in kilograms divided by height in meters squared within four categories (underweight < 18.5 kg/m^2, normal 18.5–24.9 kg/m^2, overweight 25.0–29.9 kg/m^2 or obesity ≥ 30 kg/m^2) [20], milk and milk products intake (every day 1 cup or more, less than 1 cup every day), fruit intake excluding juices (1 or more times per day, less than 1 time per day) and vegetable and salad intake excluding potato (1 or more times per day, less than 1 times per day). The independent variable also was standardized and validated by The Patient Health Questionnaire (PHQ-8) score, which was used to measure current depression on a 4-point (0–3) scale for each item. The questions were tallied to produce a total score of 0–24 points. The PHQ-8 score was treated as a continuous and as a categorical variable with 5 categories (a total score of 0 to 4 represented no significant depressive symptoms; 5 to 9 represented mild depressive symptoms; 10 to 14, moderate; 15 to 19, moderately severe; and 20 to 24, severe depressive episode). For our analysis, the current depression was defined as: a PHQ-8 score ≥ 10, and this score had the sensitivity and specificity of 88% for a diagnosis of major depression and regardless of diagnostic status, it typically represened the clinically significant depression [21, 22].

The data were analyzed by the methods of descriptive statistics, bivariate and multivariate logistic regressions. The variables were examined for each type of edentulism using the ANOVA and χ^2 analyses. Prevalence of edentulism with 95% confidence intervals (CI) adjusted on age were also obtained. In order to determine the possible predictors of edentulism, the multivariate logistic regression model was implemented for all types of edentulism. All selected independent variables were included into the model. The probability of $p < 0.05$, was taken as the minimum level of significance. The analyses were done by using the statistical software package SPSS 21, including the weight factor ("weight on"), which was used for correction of disproportionate size of the sample and adjustment of the data collected.

Results

The mean age was significantly higher among the respondents with more missing teeth. The prevalence of edentulism was the highest in the age group of 65 years and above. Only 7% of respondents who lived with a partner had no missing teeth compared to 19.1% of respondents who lived without a partner. According to the region, out of Belgrade, almost every fourth respondent was in the category of 10 or more missing teeth. The highest percentage of respondents with lower education (40.6%), one in three who were considered to be obese (32.0%), and the highest percentage of respondents with the depressive episodes, according to the PHQ-8, were in the category 10 or more missing teeth. The significant differences were observed among categories of edentulism and all independent variables except vegetable in relation to salad intake (excluding potato) (Tables 1, 2 and 3).

The highest prevalence of no missing teeth was among the respondents who lived without a partner, with high education and underweight. The prevalence rates of 1–5 missing teeth were higher than in two other categories (6–10 missing teeth, 10, or more missing teeth) in relation to all independent variables. The prevalence of all missing teeth was highest in the females, participants with lower education, inactive, underweight (BMI < 18.5 kg/m^2), and respondents with moderately severe depressive episodes (Tables 4, 5 and 6).

The results of bivariate and multivariate logistic regression analyses on the associations between edentulism and sociodemographic, life style factors and PHQ-8 scores are presented in Tables 7, 8 and 9. In the bivariate analysis, we observed that demographic factors (age, living with partner, rural areas), socioeconomic factors (lower and secondary education, unemployment, Wealth Index), life style (smoking, BMI, milk and milk products intake, fruit intake) and mental health (PHQ-8 scores and PHQ-8 depression categories) were significantly associated with edentulism, both partial and total. In the multivariate model, we found that the demographic factors (age, living with a partner), socioeconomic factors (lower and secondary education, Wealth Index), life style (smoking and BMI) and PHQ-8 score were significantly associated with edentulism. The results showed that the odds of more than 10 teeth *vs* no missing teeth were in favour of the older respondents, females, those who lived with a partner, with the lower or secondary education, the lower class, ex-smokers and smokers, the respondents with the higher BMI and the ones who belonged to the category of repondents with mild depressive symptoms (5-9 points on the scale PHQ-8).

Table 1

Sociodemographic characteristics of adult population in Serbia across categories of missing teeth

Variable (%)	Missing teeth (number)						*p*
	Total	No	1–5	6–10	10 or more	All	
Total number of patients	13,519 (100)	1,528 (11.3)	4,900 (36.2)	2,207 (15.3)	3,323 (24.6)	1,561 (11.6)	
Sex, n (%)							*
female	6,998 (51.7)	754 (10.8)	2,284 (32.6)	1,084 (15.5)	1,827 (26.1)	1,049 (15.0)	
male	6,517 (48.3)	773 (11.9)	2,616 (40.1)	1,122 (17.2)	1,495 (22.9)	512 (7.9)	
Age in years; mean ± SD	49.9 ± 17.4	31.4 ± 11.7	40.6 ± 13.2	52.6 ± 12.7	61.6 ± 12.6	68.7 ± 11.1	*
20–34	3,233 (23.9)	1,127 (34.9)	1,852 (57.3)	180 (5.6)	65 (2.0)	9 (0.3)	*
35–44	2,280 (16.8)	220 (9.6)	1,376 (60.4)	419 (18.4)	242 (10.6)	23 (1.0)	
45–54	2,379 (17.6)	87 (3.7)	886 (37.2)	644 (27.1)	627 (26.4)	135 (5.7)	
55–64	2,665 (2.1)	56 (21.0)	529 (19.8)	591 (22.2)	1,101 (41.3)	388 (14.6)	
> 65	2,960 (22.0)	38 (1.3)	257 (8.7)	372 (12.6)	1,288 (43.5)	1005 (34.0)	
Marital status, n (%)							*
living with partner	8,698 (64.3)	605 (7.0)	3,245 (37.3)	1,686 (19.4)	2,288 (26.3)	874 (10.0)	
living without partner	4,819 (35.7)	922 (19.1)	1,655 (34.3)	521 (10.8)	1,035 (21.5)	686 (14.2)	
Type of settlement, n (%)							*
urban	8027 (59.4)	1078 (13.4)	3137 (39.1)	1247 (15.5)	1685 (21.0)	880 (11.0)	
rural	5489 (40.6)	449 (8.2)	1763 (32.1)	959 (17.5)	1637 (29.8)	681 (12.4)	
Region, n (%)							*
Belgrade	3,170 (24.4)	485 (15.3)	1,247 (39.3)	437 (13.8)	649 (20.5)	352 (11.1)	
Vojvodina	3,609 (27.7)	389 (10.8)	1,249 (34.6)	638 (17.7)	875 (24.2)	458 (12.7)	
Sumadija and West Serbia	3,793 (29.0)	363 (9.6)	1,341 (35.4)	628 (16.6)	1018 (26.8)	443 (11.7)	
South and East Serbia	2,946 (18.9)	291 (9.9)	1,062 (36.0)	504 (17.1)	781 (26.5)	308 (10.5)	
Education, n (%)							*
low	3,614 (26.7)	90 (2.5)	641 (17.7)	601 (16.6)	1,469 (40.6)	813 (22.5)	
middle	7,540 (55.7)	976 (12.9)	3,141 (41.7)	1,303 (17.3)	1,520 (20.2)	600 (8.0)	
high	2,363 (17.6)	461 (19.5)	1,117 (47.3)	303 (12.8)	334 (14.1)	148 (6.3)	
Employment, n (%)							***
employed	4,857 (36.0)	734 (15.1)	2,554 (52.6)	814 (16.8)	629 (13.0)	126 (2.6)	
unemployed	3,230 (23.9)	409 (12.7)	1,442 (44.6)	569 (17.6)	658 (20.4)	152 (4.7)	
inactive	5,428 (40.1)	384 (7.1)	903 (16.6)	823 (15.2)	2035 (37.5)	1283 (23.6)	
Wealth Index, n (%)							*
poorest class	2,595 (19.1)	533 (20.5)	1,204 (46.4)	382 (14.7)	338 (13.0)	138 (5.3)	
poorer class	2,600 (19.2)	356 (13.7)	1,090 (41.9)	392 (15.1)	505 (19.4)	257 (9.9)	
middle class	2,684 (19.8)	257 (9.6)	1,034 (38.5)	471 (17.5)	602 (22.4)	320 (11.9)	
richer class	2,808 (20.7)	220 (7.8)	895 (31.9)	487 (17.3)	840 (29.9)	366 (13.0)	
richest class	2,830 (21.2)	161 (5.7)	677 (23.9)	474 (16.7)	1038 (36.7)	480 (17.0)	

SD – standard deviation; *$p < 0.05$; ***$p < 0.001$.

Table 2

The impact of life style of adult population in Serbia on categories of missing teeth

Variable expressed by the number (%) of patients	Total respondents	Missing teeth (number)					*p*
		No	1–5	6–10	10 or more	All	
Smoking status, n (%)							***
never smoked	5,577 (44.7)	728 (13.1)	2,038 (36.5)	804 (14.4)	1,296 (23.2)	711 (12.7)	
past smoker	2,326 (18.7)	202 (8.7)	802 (34.5)	437 (18.8)	598 (25.7)	287 (12.3)	
smoker	4,546 (36.5)	505 (11.1)	1,783 (39.2)	802 (17.6)	1,086 (23.9)	370 (8.1)	
BMI (kg/m^2); mean ± SD	26.6 ± 5.0	24.3 ± 4.2	25.9 ± 4.8	27.4 ± 4.9	27.8 ± 5.3	27.6 ± 5.0	***
BMI (kg/m^2), n (%)							***
underweight (< 18.5)	314 (2.4)	76 (24.2)	121 (38.5)	35 (11.1)	53 (16.9)	29 (9.2)	
normal (18.5–24.9)	4,991 (38.4)	838 (16.8)	2,100 (42.1)	661 (13.2)	949 (19.0)	443 (8.9)	
overweight (25–29.9)	4,767 (36.6)	437 (9.2)	1,699 (35.6)	828 (17.4)	1,222 (25.6)	581 (12.2)	
obesity (⩾ 30)	2,934 (22.6)	147 (5.0)	846 (28.8)	587 (20.0)	939 (32.0)	415 (14.1)	
Milk and milk products intake, n (%)							***
every day 2 or more cups	1,477 (10.9)	230 (15.6)	531 (36.0)	204 (13.8)	324 (21.9)	188 (12.7)	
every day 1 cup	5,425 (40.2)	681 (12.6)	2,068 (38.1)	830 (15.3)	1,203 (22.2)	643 (11.9)	
sometimes, not every day	6,006 (44.4)	564 (9.4)	2,122 (35.3)	1070 (17.8)	1,604 (26.7)	646 (10.8)	
never	610 (4.5)	53 (8.7)	178 (29.2)	103 (16.9)	192 (31.5)	84 (13.8)	
Fruit intake excluding juice, n (%)							**
1 or more times per day	6,157 (45.5)	664 (10.8)	2,180 (35.4)	983 (16.0)	1,555 (25.3)	775 (12.6)	
4–6 times per week	3,666 (27.1)	450 (12.3)	1,363 (37.2)	609 (16.6)	864 (23.6)	380 (10.4)	
1–3 times per week	2,770 (20.5)	308 (11.1)	1,055 (38.1)	463 (16.7)	666 (24.0)	278 (10.0)	
less than 1 time per week	760 (5.7)	92 (12.1)	251 (33.0)	120 (15.8)	194 (25.5)	103 (13.6)	
never	162 (1.2)	13 (8.0)	50 (30.9)	31 (19.1)	44 (27.2)	24 (14.8)	
Vegetable and salad intake , n (%) (excluding potato)							ns
1 or more times per day	7,776 (57.5)	843 (10.8)	2,860 (36.8)	1,226 (15.8)	1,927 (24.8)	920 (11.8)	
4–6 times per week	3,933 (29.0)	468 (11.9)	1,401 (35.6)	657 (16.7)	979 (24.9)	428 (10.9)	
1–3 times per week	1,505 (11.1)	178 (11.8)	542 (36.0)	260 (17.3)	352 (23.4)	173 (11.5)	
less than 1 time per week	219 (1.6)	28 (12.8)	74 (33.8)	41 (18.7)	51 (23.3)	25 (11.4)	
never	83 (0.8)	10 (12.0)	23 (27.7)	22 (26.5)	14 (16.9)	14 (16.9)	

BMI – body mass index; SD – standard devation; ***$p < 0.001$; **$p < 0.01$; ns – non-significant.

Table 3

Distribution of the Patient Health Questionnaire (PHQ)-8 scores of adult population in Serbia across categories of missing teeth

Variable	Missing teeth (number)						*p*
	Total	No	1–5	6–10	10 or more	All	
PHQ-8, mean ± SD	1.9 ± 3.5	0.7 ± 2.1	1.1 ± 2.3	1.8 ± 3.2	3.1 ± 4.3	3.5 ± 4.7	***
PHQ-8 score, n (%) of patients							***
none (0 to 4)	1,1591 (85.7)	1,455 (12.6)	4,577 (39.5)	1,915 (16.5)	2,515 (21.7)	1,129 (9.7)	
mild (5 to 9)	1,306 (9.6)	50 (3.8)	252 (19.3)	218 (16.7)	524 (40.1)	262 (20.1)	
moderate (10 to 14)	373 (2.8)	14 (3.8)	46 (12.3)	43 (11.5)	173 (46.4)	97 (26.0)	
moderately severe (15 to 19)	161 (1.2)	5 (3.1)	14 (8.7)	20 (12.4)	74 (46.0)	48 (29.8)	
severe (20 to 24)	86 (0.7)	3 (3.5)	10 (11.6)	11 (12.8)	37 (43.0)	25 (29.1)	

SD – standard devation; $^{***}p < 0.001$.

Table 4

Prevalence of edentulism according to the sociodemographic characteristics of adult population in Serbia

Variables (expressed by patients)	Missing teeth (number)				
	No	1–5	6–10	10 or more	All teeth
Total, % (95% CI)	10.3 (9.8–10.7)	34.3 (33.6–35.0)	16.4 (15.8–17.1)	26.2 (25.5–26.9)	12.8 (12.3–13.3)
Sex, % (95% CI)					
female	10.3 (9.6–10.9)	31.9 (30.9–32.9)***	15.6 (14.8–16.5)**	26.8 (25.8–27.7)	15.5 (14.8–16.2)***
male	10.3 (9.6–10.9)	36.9 (35.9–38.0)	17.3 (16.4–18.2)	25.6 (24.6–26.6)	9.9 (9.1–10.6)
Marital status, % (95% CI)					
living with partner	6.8 (6.2–7.4)***	36.3 (35.4–37.2)***	19.3 (18.6–20.1)***	27.0 (26.2–27.9)**	10.5 (9.9–11.1)***
living without partner	16.5 (15.8–17.3)	30.6 (29.4–31.8)	11.2 (10.2–12.2)	24.7 (23.6–25.9)	17.0 (16.1–17.9)
Type of settlement, % (95% CI)					
urban	11.8 (11.2–12.4)***	36.0 (35.1–37.0)***	15.8 (14.9–16.6)	23.3 (22.4–24.2)***	13.1 (12.4–13.8)
rural	8.2 (7.5–8.9)	32.0 (30.9–33.1)	17.3 (16.4–18.3)	30.0 (29.0–31.0)	12.4 (11.6–13.2)
Region, % (95% CI)					
Vojvodina	10.2 (9.3–11.1)***	32.0 (30.6–33.4)***	17.2 (16.0–18.4)**	25.9 (24.6–27.2)***	14.7 (13.7–15.7)***
Belgrade	13.5 (12.5–14.6)	36.6 (35.1–38.2)	13.9 (12.6–15.3)	22.6 (21.1–24.1)	13.3 (12.2–14.4)
Sumadija and West Serbia	8.6 (7.8–9.5)	33.6 (32.3–34.9)	16.9 (15.8–18.0)	28.2 (27.0–29.5)	12.6 (11.7–13.6)
South and East Serbia	9.4 (8.4–10.4)	35.5 (34.0–37.0)	17.3 (16.0–18.6)	27.3 (25.9–28.7)	10.5 (9.5–11.6)
Education, % (95% CI)					
low	9.0 (8.0–9.9)***	27.4 (26.0–28.8)***	14.9 (13.7–16.1)***	32.5 (31.2–33.9)***	16.2 (15.2–17.2)***
middle	9.1 (8.4–9.7)	35.1 (34.2–36.1)	18.1 (17.3–19.0)	25.5 (24.5–26.4)	12.2 (11.5–12.9)
high	16.4 (15.2–17.5)	43.4 (41.7–45.2)	13.6 (12.1–15.1)	17.7 (16.0–19.3)	8.9 (7.7–10.2)
Employment, % (95% CI)					
employed	6.7 (5.8–7.5)***	43.8 (42.5–45.1)***	19.2 (18.1–20.4)***	21.7 (20.5–23.0)***	8.5 (7.6–9.5)***
unemployed	4.6 (3.6–5.7)	36.0 (34.5–37.6)	20.0 (18.7–21.4)	29.0 (27.5–30.5)	10.3 (9.2–11.4)
inactive	15.9 (15.1–16.7)	26.1 (24.8–27.3)	12.4 (11.4–13.5)	28.2 (27.0–29.3)	17.4 (16.5–18.3)
Social class, % (95% CI)					
poorest class	16.0 (14.9–17.2)***	39.4 (37.7–41.1)***	15.8 (14.3–17.2)	18.9 (17.2–20.5)***	10.0 (8.7–11.2)***
poorer class	11.4 (10.3–12.5)	37.8 (36.1–39.5)	15.6 (14.1–17.0)	22.6 (21.0–24.1)	12.6 (11.5–13.8)
middle class	8.4 (7.4–9.4)	35.9 (34.3–37.5)	17.8 (16.4–19.2)	24.2 (22.7–25.7)	13.6 (12.5–14.8)
richer class	7.9 (6.9–8.9)	31.4 (29.9–32.9)	17.0 (15.7–18.3)	30.3 (28.9–31.8)	13.4 (12.3–14.5)
richest class	8.8 (7.8–9.7)	28.7 (27.2–30.2)	16.0 (14.6–17.3)	32.7 (31.3–34.2)	13.9 (12.8–14.9)

Note: Prevalence is given with 95% confidence interval (CI) adjusted on age; $^{***}p < 0.001$; $^{**}p < 0.01$.

Table 5

Prevalence of edentulism according to the life style of adult population in Serbia

Variables (expressed by patients)	Missing teeth (number)				
	No	1–5	6–10	10 or more	All
Smoking status, % (95% CI)					
never smoked	13.2 (12.4–13.9)***	36.9 (35.8–38.0)***	14.4 (13.4–15.3)***	23.1 (22.0–24.1)***	12.5 (11.7–13.2)
past smoker	10.4 (9.3–11.6)	36.3 (34.6–38.1)	18.3 (16.8–19.7)	23.8 (22.2–25.5)	11.1 (9.9–12.3)
smoker	7.0 (6.2–7.8)	32.5 (31.2–33.8)	18.4 (17.3–19.5)	29.7 (28.5–30.8)	12.5 (11.6–13.4)
BMI (kg/m^2), % (95% CI)					
underweight (< 18.5)	16.5 (13.3–19.7)***	26.2 (21.4–31.1)***	12.1 (8.0–16.3)***	27.5 (23.0–32.1)**	17.6 (14.2–21.0)***
normal BMI (18.5–24.9)	12.4 (11.6–13.2)	35.0 (33.8–36.2)	13.9 (12.9–14.9)	25.1 (23.9–26.2)	13.7 (12.8–14.5)
overweight (25–29.9)	9.8 (9.0–10.6)	35.8 (34.6–37.0)	17.4 (16.3–18.4)	25.3 (24.1–26.4)	11.9 (11.0–12.7)
obesity (⩾ 30)	7.9 (6.9–8.9)	33.3 (31.7–34.8)	19.3 (18.0–20.6)	28.2 (26.8–29.7)	11.3 (10.2–12.4)
Milk and milk products intake, % (95% CI)					
every day 2 or more cups	13.4 (12.0–14.8)***	33.0 (30.9–35.2)**	14.1 (12.3–16.0)**	24.3 (22.2–26.3)***	15.1 (13.6–16.7)**
every day 1 cup	11.4 (10.7–12.2)	35.9 (34.8–37.0)	15.6 (14.6–16.5)	23.9 (22.8–25.0)	13.2 (12.4–14.0)
sometimes, not every day	8.5 (7.8–9.2)	33.5 (32.4–34.5)	17.8 (16.9–18.7)	28.3 (27.3–29.4)	11.9 (11.1–12.6)
never	9.7 (7.6–11.9)	30.8 (27.5–34.2)	16.4 (13.5–19.3)	30.2 (27.1–33.4)	12.8 (10.4–15.2)
Fruit intake excluding juice, % (95% CI)					
1 or more times per day	10.8 (10.1–11.5)*	35.1 (34.1–36.2)**	15.9 (15.0–16.8)	25.4 (24.4–26.4)*	12.7 (12.0–13.5)*
4–6 times per week	10.6 (9.7–11.5)	34.4 (33.1–35.8)	17.0 (15.8–18.2)	25.8 (24.5–27.1)	12.1 (11.2–13.1)
1–3 times per week	8.8 (7.7–9.8)	33.6 (32.0–35.2)	16.9 (15.5–18.2)	27.8 (26.3–29.3)	12.9 (11.8–14.1)
less than 1 time per week	10.2 (8.3–12.2)	30.1 (27.0–33.1)	15.7 (13.1–18.3)	28.3 (25.4–31.2)	15.7 (13.5–17.8)
never	6.7 (2.6–10.9)	28.6 (22.1–35.0)	19.7 (14.2–25.2)	29.2 (23.1–35.3)	15.8 (11.2–20.4)
Vegetable and salad intake excluding potato, % (95% CI)					
1 or more times per day	10.1 (9.5–10.7)	35.3 (34.4–36.2)**	15.8 (15.0–16.6)**	26.0 (25.2–26.9)	12.6 (12.1–13.5)
4–6 times per week	10.8 (10.0–11.7)	33.5 (32.2–34.8)	16.9 (15.8–18.1)	26.6 (25.3–27.8)	12.2 (11.2–13.1)
1–3 times per week	9.7 (8.3–11.1)	32.4 (30.2–34.6)	17.7 (15.8–19.5)	26.3 (24.3–28.4)	13.9 (12.4–15.5)
less than 1 time per week	9.8 (6.1–13.5)	27.5 (21.8–33.2)	18.6 (13.7–23.5)	28.5 (23.1–33.9)	15.5 (11.5–19.6)
never	9.4 (3.7–15.2)	26.5 (17.7–35.3)	27.2 (19.7–34.8)	19.0 (10.7–27.4)	17.8 (11.5–24.1)

Note: Prevalence is given with 95% confidence interval (CI) adjusted on age.
BMI – body mass index; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Table 6

Prevalence of edentulism according to the Patient Health Questionnaire (PHQ)-8 scores of adult population in Serbia

PHQ-8 (depression severity categories/expressed by patients)	Missing teeth (number)				
	No	1–5	6–10	10 or more	All
None-minimal (0 to 4) % (95% CI)	10.3 (9.8–10.8)	35.7 (34.9–36.5)***	17.0 (16.3–17.7)***	24.9 (24.2–25.6)***	12.1 (11.6–12.7)***
Mild depression (5 to 9), % (95% CI)	9.3 (7.8–10.7)	27.7 (25.4–29.9)	15.3 (13.3–17.2)	32.7 (30.6–34.9)	15.1 (13.5–16.7)
Moderate depression (10 to 14), % (95% CI)	11.2 (8.4–13.9)	24.0 (19.8–28.2)	9.4 (5.8–12.9)	36.7 (32.8–40.7)	18.7 (15.8–21.7)
Moderately severe depression (15 to 19), % (95% CI)	11.8 (7.6–15.9)	22.7 (16.3–29.0)	10.1 (4.6–15.5)	34.4 (28.4–40.4)	21.1 (16.5–25.6)
Severe depression (20 to 24), % (95% CI)	12.9 (7.3–18.5)	25.9 (17.3–34.4)	9.6 (2.3–17.0)	32.4 (24.3–40.5)	19.2 (13.1–25.4)

Note: Prevalence is given with 95% confidence interval (CI) adjusted on age; *** $p < 0.001$.

Table 7

Sociodemographic factors associated with edentulism – bivariate[#] and multivariate logistic regression analysis

Variables/ Type of logistic regression analysis	1–5 missin gteeth vs. no missing teeth (4,900 vs. 1,528) OR (95% CI)	6–10 missing teeth vs. no missing teeth (2,207 vs. 1,528) OR (95% CI)	⩾10 missing teeth vs. no missing teeth (3,323 vs. 1,528) OR (95% CI)	All missing teeth vs. no missing teeth (1,561 vs. 1,528) OR (95% CI)
Age in years				
bivariate	1.07 (1.06–1.08)***	1.14 (1.13–1.15) ***	1.18 (1.17–1.19)***	1.19 (1.17–1.21)***
multivariate	1.06 (1.05–1.07)***	1.14 (1.13–1.15)***	1.18 (1.17–1.20)***	1.18 (1.16–1.20)***
Sex				
bivariate				
female	0.90 (0.80–1.02); ns	0.95 (0.80–1.13); ns	1.18 (0.96–1.45); ns	1.97 (1.45–2.67)***
male	1.00	1.00	1.00	1.00
multivariate				
female	0.98 (0.85–1.13); ns	1.20 (0.96–1.50); ns	1.42 (1.10–1.84)**	1.83(1.25–2.67)**
male	1.00		1.00	1.00
Marital status				
bivariate				
living with partner	1.87 (1.64–2.14)***	2.54 (2.09–3.07) ***	1.99 (1.59–2.48) ***	1.35 (0.98–1.86); ns
no partner	1.00	1.00	1.00	1.00
multivariate				
living with partner	1.58 (1.36–1.84) ***	2.14 (1.70–2.69) ***	1.99 (1.51–2.63) ***	1.34 (0.90–2.00); ns
no partner	1.00	1.00	1.00	1.00
Type of settlement				
bivariate				
urban	1.00	1.00	1.00	1.00
rural	1.44 (1.27–1.65)***	2.26 (1.87–2.72)***	2.62 (2.11–3.26) ***	2.11 (1.53–2.90)***
multivariate				
urban	1.00	1.00	1.00	1.00
rural	1.00 (0.84–1.91); ns	1.06 (0.81–1.38); ns	1.09 (0.80–1.50); ns	1.01 (0.64–1.59); ns
Education				
bivariate				
low	2.79 (2.15–3.60) ***	11.03 (7.79–15.63)***	18.10 (12.35–26.51) ***	10.06 (6.18–16.37) ***
middle	1.76 (1.51–2.02) ***	4.52 (3.54–5.76) ***	5.81 (4.36–7.74) ***	5.51 (3.66–8.30) ***
high	1.00	1.00	1.00	1.00
multivariate				
low	1.98 (1.45–2.69) ***	4.89 (3.18–7.51) ***	5.45 (3.37–8.81) ***	4.20 (2.23–7.93) ***
middle	1.54 (1.31–1.81) ***	3.06 (2.32–4.030) ***	3.29 (2.37–4.56) ***	3.41 (2.08–5.59) ***
high	1.00	1.00	1.00	1.00

Table 7 (continued)

Variables / Type of logistic regression analysis	1–5 missing teeth vs. no missing teeth (4,900 vs. 1,528) OR (95% CI)	6–10 missing teeth vs. no missing teeth (2,207 vs. 1,528) OR (95% CI)	⩾10 missing teeth vs. no missing teeth (3,323 vs. 1,528) OR (95% CI)	All missing teeth vs. no missing teeth (1,561 vs. 1,528) OR (95% CI)
Employment				
bivariate				
employed	1.00	1.00	1.00	1.00
unemployed	1.23 (1.07–1.42) **	1.73 (1.40–2.12)***	2.34 (1.84–2.97)***	2.44 (1.66–3.60)***
inactive	0.60 (0.51–0.71)***	0.73 (0.56–0.93) *	1.12 (0.84–1.49); ns	2.22 (1.52–3.25)***
multivariate				
employed	1.00	1.00	1.00	1.00
unemployed	1.06 (0.90–1.24); ns	1.08 (0.84–1.40); ns	1.23 (0.91–1.67); ns	1.44 (0.90–2.30); ns
inactive	0.60 (0.50–0.73) ***	0.50 (0.36–0.69) ***	0.72 (0.50—1.04); ns	1.43 (0.89–2.31); ns
Wealth Index				
bivariate				
poorest class	1.97 (1.60–2.44) ***	5.61 (4.14–7.61) ***	10.52 (7.40–14.97)***	9.39 (5.53–15.94)***
poorer class	2.02 (1.67–2.44)***	4.14 (3.12–5.49)***	7.37 (5.27–10.31)***	6.44 (3.95–10.52)***
middle class	1.89 (1.58–2.26)***	3.20 (2.43–4.21)***	4.00 (2.86–5.60)***	6.68 (4.10–10.89)***
richer class	1.41 (1.20–1.67)***	1.88 (1.43–2.45)***	2.74 (1.97–3.80) ***	3.71 (2.29–6.03)***
eichest class	1.00	1.00	1.00	1.00
multivariate				
poorest class	1.44 (1.08–1.91)*	3.01 (1.96–4.61)***	4.88 (2.95–8.08) ***	5.21 (2.49–10.91)***
poorer class	1.59 (1.26–2.01) ***	2.48 (1.72–3.57)***	3.73 (2.42–5.75)***	3.15 (1.66–5.98) **
middle class	1.56 (1.27–1.91)***	1.96 (1.40–2.73)***	2.52 (1.69–3.76)***	3.65 (2.05–6.51)***
richer class	1.27 (1.06–1.53) **	1.48 (1.09–2.01) *	2.01 (1.38–2.91)***	2.27 (1.28–4.02) **
richest class	1.00	1.00	1.00	1.00

[#]Adjusted on age.
OR – odds ratio; CI – confidence interval.
[***]$p < 0.001$; [**]$p < 0.01$; [*]$p < 0.05$; ns – non-significant.

Table 8

Life style factors associated with edentulism – bivariate# and multivariate logistic regression analysis

Variables (expressed by patients)/ Type of logistic regression analysis	1–5 missing teeth vs. no missing teeth (4,900 vs. 1,528) OR (95% CI)	6–10 missing teeth vs. no missing teeth (2,207 vs. 1,528) OR (95% CI)	⩾10 missing teeth vs. no missing teeth (3.323 vs. 1,528) OR (95% CI)	All missing teeth vs. no missing teeth (1,561 vs. 1,528) OR (95% CI)
Smoking status				
bivariate				
never smoked	1.00	1.00	1.00	1.00
past smoker	1.07 (0.88–1.29); ns	1.46 (1.12–1.90) **	1.33 (0.97–1.83); ns	1.02 (0.66–1.57); ns
smoker	1.27 (1.10–1.45) **	2.04 (1.66–2.51); ***	3.16 (2.46–4.07); ***	1.76 (1.23–2.53) **
multivariate				
never smoked	1.00	1.00	1.00	1.00
past smoker	0.96 (0.79–1.17); ns	1.33 (0.99–1.79); ns	1.43 (1.00–2.03) *	1.13 (0.69–1.85); ns
smoker	1.09 (0.94–1.26); ns	1.60 (1.26–2.02) ***	2.80 (2.08–3.76); ***	1.74 (1.14–2.65) **
BMI				
bivariate	1.03 (1.02–1.05)***	1.06 (1.04–1.08)***	1.04 (1.01–1.07)**	1.04 (1.00–1.08)**
multivariate	1.02 (0.99–1.03); ns	1.05 (1.02–1.07)***	1.05 (1.00–1.09)*	1.03 (0.97–1.10); ns
BMI (kg/m^2) in categories				
bivariate				
underweight (< 18.5)	0.84 (0.62–1.15); ns	1.25 (0.75–2.07); ns	1.15 (0.59–2.22); ns	1.17 (0.37–3.67); ns
normal BMI (18.5–24.9)	1.00	1.00	1.00	1.00
25 and more	1.16 (1.02–1.32)*	1.55 (1.29–1.86)***	1.23 (0.99–1.52); ns	1.24 (0.90–1.71); ns
multivariate				
underweight (< 18.5)	1.00 (0.70–1.41); ns	1.23 (0.65–2.35); ns	1.12 (0.49–2.58); ns	1.32 (0.31–5.59); ns
normal BMI (18.5–24.9)	1.00	1.00	1.00	1.00
25 and more	0.82 (0.66–1.02); ns	0.91 (0.65–1.27); ns	0.85 (0.57–1.26); ns	1.02 (0.58–1.81); ns
Milk and milk products intake				
bivariate				
every day 1 cup or more	1.00	1.00	1.00	1.00
less than every day 1 cup	1.26 (1.11–1.43) ***	1.76 (1.47–2.10) ***	1.75 (1.43–2.15) ***	1.39 (1.03–1.87) *
multivariate				
every day 1 cup or more	1.00	1.00	1.00	1.00
less than every day 1 cup	1.09 (0.95–1.25); ns	1.16 (0.94–1.43); ns	1.21 (0.94–1.55); ns	1.09 (0.76–1.56); ns
Fruit intake excluding juice				
bivariate				
1 or more times per day	1.00	1.00	1.00	1.00
less than 1 time per day	1.14 (1.02–1.29)*	1.46 (1.21–1.74)***	1.48 (1.20–1.82)***	1.25 (0.92–1.68); ns
multivariate				
1 or more times per day	1.00	1.00	1.00	1.00
less than 1 time per day	1.07 (0.91–1.25); ns	1.19(0.93–1.53); ns	1.18 (0.87–1.59); ns	1.10 (0.72–1.69); ns

Table 8 (continued)

Variables (expressed by patients)/ Type of logistic regression analysis	1–5 missing teeth vs. no missing teeth (4,900 vs. 1,528) OR (95% CI)	6–10 missing teeth vs. no missing teeth (2,207 vs. 1,528) OR (95% CI)	⩾10 missing teeth vs. no missing teeth (3,323 vs. 1,528) OR (95% CI)	All missing teeth vs. no missing teeth (1,561 vs. 1,528) OR (95%CI)
Vegetable and salad intake excluding potato and juice				
bivariate				
1 or more times per day	1.00	1.00	1.00	1.00
less than 1 times per day	1.02 (0.89–1.14); ns	1.30 (1.09–1.56) **	1.14 (0.92–1.40); ns	1.09 (0.80–1.48); ns
multivariate				
1 or more times per day	1.00	1.00	1.00	1.00
less than 1 times per day	0.90 (0.77–1.05); ns	1.13 (0.88–1.45); ns	1.04 (0.77–1.41); ns	1.10 (0.71–1.70); ns

#Adjusted on age.

BMI – body mass index; OR – odds ratio; CI – confidence interval; ***$p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns–non-significant.

Table 9

PHQ-8 scores and categories associated with dentulism – bivariate# and multivariate logistic regression analysis

Variables/ Type of logistic regression analysis	1–5 missing teeth vs. no missing teeth (4,900 vs. 1528) OR (95% CI)	6–10 missing teeth vs. no missing teeth (2,207 vs. 1,528) OR (95% CI)	⩾10 missing teeth vs. no missing teeth (3,323 vs.1,528) OR (95% CI)	All missing teeth vs. no missing teeth (1,561 vs.1,528) OR (95% CI)
PHQ-8 depression score				
bivariate	1.04 (1.01–1.08)*	1.11 (1.07–1.15) ***	1.13 (1.08–1.17) ***	1.10 (1.05–1.15) ***
multivariate	1.03 (0.99–1.06); ns	1.08 (1.04–1.13) ***	1.06 (1.01–1.11)**	1.03 (0.97–1.08); ns
bivariate				
not currently depressed (0 to 4)	1.00	1.00	1.00	1.00
depressive symptoms (5–9)	1.12 (0.81–1.56); ns	1.78 (1.19–2.66)**	2.62 (1.73–3.98)***	1.91 (1.11–3.25) *
depressive episodes (10 to 24)	0.60 (0.36–0.99) *	1.60 (0.88–2.90); ns	1.90 (1.09–3.33)*	2.04 (1.03–4.05) *
multivariate				
not currently depressed (0 to 4)	1.00	1.00	1.00	1.00
depressive symptoms (5–9)	1.11 (0.75–1.57); ns	1.47 (0.93–2.32); ns	1.68 (1.02–2.77)*	1.08 (0.57–2.04); ns
depressive episodes (10 to 24)	0.58 (0.33–1.02); ns	1,59 (0.80–3.13); ns	1.03 (0.52–2.02); ns	1.19 (0.52–2.72); ns

#Adjusted on age.

OR – odds ratio; CI – confidence interval; ***$p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns – non-significant.

Discussion

The WHO data indicated that 15%–20% of the world midlife population (age 35–44 years) is at risk of tooth loss, and about 30% of the population aged 65–74 years has no teeth [23]. In the adult population aged 20 years, or older, the prevalence of complete loss of teeth is the lowest in Canada [24] and Mexico [25] (around 6%) while in Serbia it is almost two times higher (11.3%). Since 2000, the trend of complete tooth loss in the population of Serbia is in decline while the situation with the loss of 10, or more teeth, reversed [26].

It is noticed that women were at a greater risk of partial (10 or more missing teeth), or complete edentulism. The reasons for the increased chance for women to partially or completely lose their teeth are still unsolved and there are only assumptions [27]. Perhaps we should pay more attention to the period of pregnancy in women when there is a hormonal imbalance causing the teeth and gums become more sensitive to bacteria, which increase the chance of infection and tooth loss.

Examining the connection between marital status and a lack of teeth, we could observe that a life with a partner was in correlation with the partial lack of teeth (1-5, 6-10 and 10 teeth and more teeth, but not all). The respondents who lived with a partner had more than two times higher chance of a partial lack of teeth compared to those who lived alone. Conversely, the results of research carried out in Canada [28] and Sweden [29] indicated that life without a partner is a risk factor for tooth loss. This finding may be explained by poor relations between partners, socioeconomic situation and the lack of support.

Analyzing the socioeconomic factors, it was registered that there was a significant association between lower level of education and the Wealth Index with a partial and a complete lack of teeth. However, when we observed unemployment as a socioeconomic indicator, we noted that the inactive population inversely correlated with partial missing of teeth and had 40% lower chances of missing 1-5 teeth *vs* no missing teeth. This result could be explained by the structure of the inactive population, since the majority of respondents in this category are students and housewives who are younger, or middle age.

The respondents with the mild depressive symptoms were more likely to lose 10, or more teeth compared to those who were not depressed, which was in line with the increase in PHQ-8 score. However, Hybels et al. [30] did not confirm an association between the symptoms of depression and the lack of teeth. The depression symptoms can affect oral health in two ways. The first is by biological mechanism, by contributing to problems with the immune system. Depression can stimulate the production of proinflammatory cytokines that can influence the conditions that contribute to the development of periodontal disease [31]. Also, the use of certain antidepressants is associated with hyposalivation, which can affect the poor state of oral health, including dry mouth, burning sensation, the occurrence of periodontal disease and caries [32]. Another way is the impact of depression symptoms on the insufficient motivation for oral hygiene practice, and reduced frequency of tooth brushing and irregular dental visits [6]. Based on the perceived situation, we can say that the maintenance of depressive disorders under control, regular visits to the dentist and social support contribute to the prevention of tooth loss.

Smoking is a significant predictor of tooth loss [3]. Since 2000, the percentage of smokers in Serbia decreased significantly to the value of 36.5% in 2013 [17]. Compared to the prevalence of smoking in the European countries, we can say that Serbia is approaching the countries of Southeastern Europe with the highest prevalence (Greece 38.9%, Croatia 35%, Bulgaria 29.2%) compared to the countries of Northern Europe with the lowest prevalence (Belgium 18%, UK 19%) [33]. Regardless of the geographical position, this situation can be viewed from the aspect of history, tradition and culture.

Our study had several limitations. First, the independent variables including tooth loss are self-reported with possible bias. Second, we were unable to examine other factors associated with tooth loss, such as dental insurance, dental caries, periodontal disease, community water fluoridation, or antidepressant medications. Third, our study was cross-sectional. Therefore, we can not infer causality. The cross-sectional studies are not relational, and cannot determine causal relationships between the different variables. Mental health disorders coexisting with poor oral health, or other chronic conditions, contribute to the severity and progression of disease and poorer outcomes [34]. Conversely, the persons with tooth loss may have a lower socioeconomic status, lower self-esteem, lack of access to oral health services; also, they may practice other health-compromising behaviors, or have other health conditions that require greater resources and management. These factors may lead to depression, or contribute to the severity of depression as well [6]. It should be noted that in addition to the demographic, socioeconomic factors and lifestyle, there are other factors that are known or suspected to affect the state of oral health that could be subject to examination in future research. These are psychological factors (fear, stressful life events, psychosocial issues) and factors related to the community (relations between people, social support).

Conclusion

This study demonstrated that the sociodemographic and lifestyle factors and depressive symptoms are also important factors related to the tooth loss. The results from this study could serve as a platform for the health policy decision – making, planning and organizing health care in the field of oral health, and to promote the need for better accessibility to dental care as well as for the adoption of specific health promotion programs that can improve oral health of vulnerable categories of the population.

Acknowledgements

The 2013 Serbian National Health Survey is the third national health survey carried out and financed by the Ministry of Health of the Republic of Serbia. We are grateful for the assistance of the Ministry of Health of the Republic of Serbia and the Institute of Public Health of Serbia „Dr Milan Jovanovic-Batut“, who authorized the use of the database for this study.

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Received on October 11, 2017.
Revised on November 9, 2017.
Accepted on November 21, 2017.
Online First December, 2017.

ORIGINAL ARTICLE
UDC: 616.895.7-08
https://doi.org/10.2298/VSP171002191S

Factors associated with adherence to guidelines of good clinical practice during hospital treatment of patients with the first episode of schizophrenia spectrum disorders

Faktori povezani sa poštovanjem preporuka dobre kliničke prakse tokom bolničkog lečenja bolesnika sa prvom epizodom oboljenja iz shizofrenog spektra

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Abstract

Background/Aim. Adherence to the guidelines of Good Clinical Practice (GCP) during the treatment of specific disorders is considered a guarantee of the implementation of a uniform, evidence-based clinical practice in psychiatry. The aim of this study was to analyze the concordance of prescribing patterns of antipsychotic drugs with the recommendations of good clinical practice in Serbia and an insight into the effects that introduction of the National Guideline for the Diagnosis and Treatment of Schizophrenia had on the prescribing practice in this area. **Methods.** Non-interventional, observational study was conducted at the Clinic for Mental Disorders "Dr Laza Lazarević" in Belgrade. It included a consecutive sample of 675 previously untreated patients, hospitalized from January 1st, 2012 to December 31st, 2015, dismissed with a discharge diagnosis of any of schizophrenia spectrum disorders. The data about demographic and clinical characteristics of patients, characteristics of prescribers and prescribed antipsychotics were obtained retrospectively, from the patients' medical records. For the analysis of primary data, the descriptive statistical methods and methods for testing statistical hypotheses were used. A method of logistic regression was used to identify the factors associated with adherence to the GCP recommendations. **Results.** Totaly, 446 (66.1%) of subjects were treated with antipsychotic monotherapy. After the introduction of National Guideline for the Diagnosis and Treatment of Schizophrenia prescribing of second generation antipsychotic monotherapy (78.41% vs. 63.5%, respectively; $p < 0.001$) increased significantly compared to the previous period. The factors independently associated with adherence to the recommendations of the GCP were the year of hospitalization, the age of the prescriber, and the age and education of the patient. **Conclusion.** After the introduction of the National Guide to Good Clinical Practice for the Diagnosis and Treatment of Schizophrenia there have been significant, but insufficient changes in the prescribing patterns of antipsychotics during the treatment of the first psychotic episode in Serbia.

Key words:
practice guidelines as topic; guideline; serbia; schizophrenia; drug therapy; antipsychotic agents.

Apstrakt

Uvod/Cilj. Poštovanje smernica datih u vodičima dobre kliničke prakse tokom lečenja specifičnih poremećaja smatra se garancijom sprovođenja uniformne, na dokazima zasnovane kliničke prakse u psihijatriji. Cilj ovog rada bio je da se analizira usklađenost obrazaca propisivanja antipsihotičkih lekova sa preporukama dobre kliničke prakse u Srbiji i da se ostvari uvid u efekte koje je na propisivačku praksu u ovoj oblasti imalo donošenje Nacionalnog vodiča dobre kliničke prakse za dijagnostikovanje i lečenje shizofrenije. **Metode.** Neinterventna, opservaciona studija sprovedena je na Klinici za psihijatrijske bolesti "Dr Laza Lazarević" u Beogradu. Istraživanje je obuhvatilo uzastopni uzorak od 675 prethodno netretiranih bolesnika, lečenih hospitalno u periodu od 1. januara 2012. godine do 31. decembra 2015. godine, otpuštenih sa dijagnozom nekog od poremećaja iz shizofrenog spektra. Demografski podaci i podaci o kliničkim karakteri-

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stikama bolesnika, karakteristikama propisivača i propisanim antipsihoticima sakupljeni su retrospektivno, uvidom u medicinsku dokumentaciju bolesnika. Za analizu primarnih podataka korišćene su deskriptivne statističke metode i metode za testiranje statističkih hipoteza. Metoda logističke regresije korišćena je za identifikaciju faktora povezanih sa poštovanjem preporuka dobre kliničke prakse. **Rezultati.** Antipsihotičnom monoterapijom tretirano je 446 (66,1%) ispitanika. Nakon donošenja Nacionalnog vodiča dobre kliničke prakse za dijagnostikovanje i lečenje shizofrenije, propisivanje antipsihotične monoterapije antipsihoticima druge generacije bilo je značajno češće u odnosu na prethodni period (78,41% prema 63,5%; $p < 0.001$). Faktori nezavisno povezani sa poštovanjem preporuka dobre kliničke prakse su bili: starost ordinirajućeg lekara, starost i obrazovanje bolesnika i godina hospitalizacije. **Zaključak.** Nakon donošenja Nacionalnog vodiča dobre kliničke prakse za dijagnostikovanje i lečenje shizofrenije došlo je do značajnih, ali nedovoljnih promena u obrascima propisivanja antipsihotičke terapije tokom tretmana prve psihotične epizode u Srbiji.

Ključne reči:
lekarska praksa, oblici; vodiči; srbija; shizofrenija; lečenje lekovima; antipsihotici.

Introduction

Schizophrenia is a severe, chronic mental disorder that is among the twenty leading global causes of disability in the world [1]. The course and outcome of the earliest stages of schizophrenia have a pathoplastic effects on its further course [2]. It has been proven that the time period between the onset of the first psychotic symptoms and the initiation of treatment (the duration of untreated psychosis – DUP) correlates with the prolongation of the time needed to achieve remission and is associated with a poor response to treatment [3–5]. The first psychotic episode (FEP) is a critical developmental transition that may affect a further course of schizophrenia, possibly by induction of neurotoxic changes in neural networks, thus leading to disease chronicity [6].

With each subsequent relapse, further deterioration occurs in the psychopathological, neurocognitive and functional domain and the time required for achieving remission is prolonged, and the possibility for good treatment response decreases [7–9]. It is considered that a critical period of 2 to 5 years after the onset of psychosis determines the functional outcome of the disease and during this period therapeutic interventions have a maximum effect [10]. Therefore, early detection, adequate treatment and the achievement of a full symptomatic and functional recovery from FEP, maintenance of adherence to the treatment and prevention of relapse in early stages of the disease are the main goals of the treatment of schizophrenia [11–14].

Over the past two decades, the adherence to the guidelines of Good Clinical Practice (GCP) during the treatment of specific disorders, is considered a guarantee of the implementation of a uniform, evidence-based clinical practice in psychiatry [15]. For more than 15 years, GCP guidelines have been applied worldwide for the diagnosis and treatment of schizophrenia [16–18]. In Serbia, at the end of 2013, the Republic Commission for the Development and Implementation of Guidelines in Clinical Practice developed the National Guidelines of GCP for the Diagnosis and Treatment of Schizophrenia [19]. As with most other guidelines, the choice of antipsychotics (AP) is primarily based on the adverse reaction profile, since their efficacy, with the exception of clozapine, is considered practically equal. An assessment of the previous response to therapy, the presence of comorbid psychiatric and somatic diseases, the use of concomitant medication, safety in overdose and, of course, drug availability is recommended when choosing antipsychotic for the initial treatment of acute episode. The recommendation is to use one of the second generation antipsychotics (SGAs), except for clozapine, as the first-line therapy, whereas the first generation antipsychotics (FGAs) may be used as the first-line therapy only in the patients who had multiple episodes with previously favorable response and lack of significant side effects.

In the case of the first episode, the use of SGAs as the first treatment line is mandatory and it is necessary to follow the recommendation on the use of lower doses (dose from the lower part of the therapeutic range), since previously untreated patients are more sensitive to the development of adverse reaction and show a good response to lower doses of antipsychotics. Unlike most international guidelines, the Serbian treatment guideline for completely, or partially non-adherent patients recommends the use of long-acting APs in the case of FPE as well.

It is reasonable to assume that in recent years, following the introduction of the National Guideline for the Diagnosis and Treatment of Schizophrenia and the inclusion of a significant number of SGA into the List of Medicines covered by a mandatory health insurance, there has been a significant change in the prescribing patterns in everyday clinical practice in Serbia.

The aim of this study was to analyze the concordance of prescribing patterns of antipsychotic drugs with the recommendations of good clinical practice in Serbia. This analysis covered the period from 2012 to 2015 which enables a comparison of drug prescribing patterns before and after the introduction of the National Guideline for the Diagnosis and Treatment of Schizophrenia, and an insight into the effects that its introduction had on the prescribing practice in this area.

Methods

This observational cross-sectional study was conducted at the Clinic for Psychiatric Disorders "Dr Laza Lazarević" in Belgrade and it included 675 subjects. A consecutive sample included all previously untreated patients, hospitalized in the period from January 1st, 2012 to December 31st, 2015 that had been dismissed according to the International Statistical Classification of Diseases and Related Health Problems (ICD 10) discharge diagnosis with: Schizophrenia (F20.0-

F20.9), Acute polymorphic psychotic disorder with symptoms of schizophrenia (F23.1), Acute psychotic disorder similar to schizophrenia (F23.2), Schizoaffective disorder (F23.2) and Unspecified nonorganic psychosis (F29) [20].

The subjects were divided into two groups: a group discharged during 2012 and 2013 and a group discharged in 2014 and 2015. The timeline was selected because at the end of 2013, the National Guideline for the Diagnosis and Treatment of Schizophrenia was published in Serbia.

Beside above mentioned diagnostic categories, the inclusion criteria were: over 18 years of age and the first psychotic episode, defined as the first contact with antipsychotic treatment [21]. The exclusion criteria were: any previous antipsychotic treatment; diagnosis of bipolar disorder, psychotic depression, psychotic disorder caused by the use of psychoactive substances (PAS), psychotic disorder due to the general medical condition; the presence of primary and secondary cognitive deficit.

The research was approved by the Ethics Committee of the Clinic for Psychiatric Disorders "Dr Laza Lazarević". The data were obtained from the patients' medical records, including the following: demographic data, type and duration of hospitalization in days, duration of untreated psychosis in months, somatic comorbidity, suicidality, use of PAS, antipsychotics prescribed at discharge, and characteristics of prescribers (age and years of experience as a specialist). DUP is defined as the time interval between the onset of the first clear psychotic symptoms and the first prescription of antipsychotics and estimated based on the data from the history of the disease, obtained from the patients and their relatives [4, 5, 22]. Suicidality is defined as presence of suicidal ideas, intentions or attempts, noted during the psychiatric assessment at addmision. The presence of PAS was assessed on the basis of data obtained from the patients, their relatives and the qualitative urine tests conducted immediately after admission to hospital and it was related to the actual exposure. The analysis of antipsychotic therapy, prescribed to the discharged patients, included the class and type of prescribed antipsychotics. Antipsychotic polypharmacy is defined as the simultaneous prescription of at least two different antipsychotics. The adherence to the recommendations of GCP was assessed for each patient individually, following an analysis of the prescribed regimen of antipsychotic therapy and its compliance with the recommendations of GCP for the treatment of the first psychotic episode. For the analysis of primary data, the descriptive statistical methods and methods for testing statistical hypotheses were used. Of the descriptive statistical methods, the measures of central tendency (arithmetic mean, median), measurements of variability (standard deviation) and relative numbers were used. For testing the relationship between the categorical variables, the χ^2 test was used. The Fisher's test was used instead of the χ^2 test when any expected frequency was less than 1, or 20% of expected frequencies were less than, or equal to 5. For the comparison of continuous variables, the Student's *t*-test and Mann-Whitney test were used. The statistical hypotheses on differences were tested at a statistical significance level of 0.05. The method of logistic regression was used to identify the factors associated with the adherence to the GCP recommendations. We analysed the association of the sociodemographic and clinical variables related to the patients and prescribers with the outcome variable (GCP recommendation) Initially, the variables potentially associated with the outcome were analyzed by the univariate logistic regression model. The variables significant at $p < 0.05$ in the univariate logistic regression were included in the multiple logistic regression model in order to identify the factors independently associated with the adherence to GCP recommendations. We used the multicollinearity analysis to check collinearity among the explanatory variables. Multicollinearity was assessed using a variance inflation factor (VIF). The VIF higher than 5 indicated the highly correlated variables and presence of multicollinearity. All statistical analyses were carried out using the statistical program SPSS 24.0 (SPSS, Inc., Chicago, IL, USA).

Results

This study included a consecutive sample of 675 patients treated for the first time in the period from 2012 to 2015 with the diagnosis of schizophrenia and other schizophrenia–spectrum disorders. In the period 2012–2013, 330 patients (48.9% of the total sample) were discharged from hospital, whereas in 2014 and 2015 a total of 345 patients (51.1%) were discharged after treatment of the first episode of schizophrenia spectrum disorders.

The sociodemographic characteristics of patients are shown in Table 1. A majority of subjects (60.7%) were males. The average age was 28.5 ± 8.7 years. The youngest subject was 18, and the oldest one 57. Most of them had secondary education, were unemployed and single. More than two-thirds of them lived with their parents. There were no statistically significant differences between the groups, except that those who were discharged during 2014 and 2015 were more often unemployed and lived with their parents than those who were discharged during 2012 and 2013.

Of all patients who had been discharged from psychiatric hospital care 34.7% were diagnosed with schizophrenia and 34.1% with unspecified psychoses, while the schizoaffective disorder was diagnosed in only 3.6% of patients. A statistically significant difference was found between the groups. The patients dismissed from hospital in the period 2014–2015 were more often discharged with the diagnosis of acute psychotic disorder, while the frequency of diagnosis of schizophrenia declined ($\chi^2 = 37.013$; $p < 0.001$).

Other clinical characteristics of patients are shown in Table 2. No statistically significant differences were found between the groups in any clinical feature.

FGA was prescribed to a total of 410 (60.7%) subjects, while SGA was prescribed to 504 (74.7%). A statistically significant difference was found between the groups. During 2014 and 2015, the prescription of FGAs decreased significantly, while the frequency of prescribing SGA increased significantly (Table 3).

Table 1

The sociodemographic characteristics of patients

Parametres	Patients (n = 675)	2012/2013 (n = 330)	2014/2015 (n = 345)	χ^2/t/Z	*p*
Gender, n (%)					
male	410 (60.7)	202 (61.2)	208 (60.3)	0.806	0.814
female	265 (39.3)	128 (38.8)	137 (39.7)		
Age (years), mean ± SD	28.5 ± 8.7 (18–57)	28.6 ± 8.5 (18–54)	28.5 ± 8.8 (18–57)		
Education, n (%)					
primary	135 (20.0)	68 (20.6)	67 (19.4)		
secondary	430 (63.7)	207 (62.7)	223 (64.6)	0.270	0.874
university	110 (16.3)	55 (16.7)	55 (15.9)		
Employment, n (%)					
employed	80 (11.9)	34 (10.3)	46 (13.3)		
unemployed	491 (72.7)	256 (77.6)	235 (68.1)	8.505	0.037
retired	1 (0.1)	0 (0.0)	1 (0.3)		
students	103 (15.3)	40 (12.1)	63 (18.3)		
Marital status, n (%)					
single	557 (82.5)	276 (83.6)	281 (81.4)		
married	86 (12.7)	42 (12.7)	44 (12.8)	1.774	0.621
divorced	27 (4.0)	10 (3.0)	17 (4.9)		
widowed	5 (0.7)	2 (0.6)	3 (0.9)		
Household, n (%)					
alone	70 (10.4)	43 (13.0)	27 (7.8)		
with parents	495 (73.3)	225 (68.2)	270 (78.3)	9.210	0.027
with partner	83 (12.3)	47 (14.2)	36 (10.4)		
other	27 (4.0)	15 (4.5)	12 (3.5)		

χ^2 – chi-square test; t – *t*-test; Z – Mann-Whitney test; SD – standard deviation.

Table 2

Clinical characteristics of patients

Parameters	Total number of patients (n = 675)	2012/2013 addmission (n = 330)	2014/2015 addmission (n = 345)	χ^2/t/Z	*p*
Type of hospital admmision, n (%)					
involuntary	160 (23.7)	76 (23.0)	84 (24.3)	0.162	0.718
voluntary	515 (73.6)	254 (77.0)	261 (75.7)		
Police assistance, n (%)					
yes	362 (53.6)	165 (50.0)	197 (57.1)	0.064	0.076
no	313 (46.4)	165 (50.0)	148 (42.9)		
Duration of hospitalization (days)					
mean ± SD	36.5 ± 18.6	37.4 ± 19.8	35.7 ± 17.5	-1.348	0.178
range	(1–116)	(1–109)	(1–116)		
Duration of untreated psychosis (DUP), months					
mean ± SD	31.4 ± 49.8	27.7 ± 42.0	34.8 ± 56.1	-0.569	0.569
range	(1–348)	(1–348)	(1–333)		
Suicidality, n (%)					
no	539 (79.7)	266 (80.6)	273 (79.1)		
ideas and intentions	79 (11.7)	33 (10.0)	46 (13.3)	2.337	0.311
suicide attempt	57 (8.4)	31 (9.4)	26 (7.5)		
Substance use, n (%)					
yes	192 (28.4)	86 (26,1)	106 (30.7)	1.803	0.201
no	483 (71.6)	244 (73.9)	239 (69.3)		

χ^2 – chi-square test; *t* – Student *t*-test; Z – Mann-Whitney test; SD – standard deviation.

Table 3

Frequency of prescribing FGAs and SGAs

Antipsychotics	Total number of patients (n = 675)	2012/2013 admissions (n = 330)	2014/2015 admissions (n = 345)	χ^2/t/Z	*p*
FGAs, n (%)	410 (60.7)	229 (69.3)	181 (52.5)	20.274	< 0.001
SGAs, n (%)	504 (74.6)	218 (66.1)	286 (82.9)	25.281	< 0.001

FGA – first generation antipsychotics; SGA – second generation antipsycotichs.

Out of a total of 675 subjects, 446 (66.1%) of them were treated with antipsychotic monotherapy (AMT). Out of 229 (33.9%) of subjects treated with antipsychotic polypharmacy, 218 (32.3% of the total sample) were discharged with two antipsychotics in regular therapy, while 11 of them (1.6% of the total sample) were discharged with more than two antipsychotics prescribed. There were no statistically significant differences in the frequency of prescribing antipsychotic monotherapy and antipsychotic polypharmacy among the patient groups treated in 2012/2013 and 2014/2015. Out of total of 446 subjects treated with monotherapy, FGA was prescribed to 129 (28.9%), and SGA to 317 (71.1%). A statistically significant difference was found between the groups in the frequency of prescribing different classes of antipsychotic as a monotherapy. During 2014 and 2015, the prescription of SGA as antipsychotic monotherapy increased significantly, while the frequency of prescribing FGA decreased significantly (Table 4).

There was the adherence to good clinical practice recommendations for the treatment of schizophrenia in a total of 227 (33.6%) of patients, with 94 (28.5%) patients discharged during 2012 and 2013, and 133 (38.6%) discharged during 2014 and 2015. The difference in the frequency of compliance with the recommendation of good clinical practice between the groups was statistically significant (χ^2 = 7.657; p = 0.007).

The variables statistically significant associated with the adherence to the recommendations of good clinical practice in the univariate logistic regression analysis were included in the multivariate model (Table 5). The variable "Experience as a specialist" was highly correlated with the variable "Age of doctors" (VIF = 5.112), and was removed from the multivariate model.

The factors independently associated with the adherence to the good clinical practice recommendations were the age of the psychiatrist, age and education of patient, and year of hospitalization (Table 5). With every year more of patients' age, the adherence to the good clinical practice recommendations decreased by 4%. The psychiatrists younger than 46 years of age more often complied with the recommendations of good clinical practice than those over 46 years of age. When treating the patients with secondary education and university degree, the psychiatrist adhered to the recommendations of the GCP 1.9 times more often than when treating those with the primary school. Also, the adherence to the recommendations of good clinical practice was 1.5 times more often in the patients treated in 2014 and 2015 than in those treated in 2012 and 2013.

Table 4

Frequency of prescribing the antipsychotic monotherapy (FGA and SGA) and antipsychotic polypharmacy

Parametres	Total number of patients (n = 675)	2012/2013 admissions (n = 330)	2014/2015 admissions (n = 345)	χ^2	p
AMT, n (%)	446 (66.1)	219 (66.4)	227 (65.8)	0.195	0.907
FGAs, n (%)	129 (28.9)	80 (36.53)	49 (21.59)	12.108	
SGAs, n (%)	317 (71.1)	139 (63.47)	178 (78.41)		
APP, n (%)	229 (33.9)	111 (33.6)	118 (34.2)		
2AP, n (%)	218 (32.3)	105 (31.8)	113 (32.8)		
> 2AP, n (%)	11 (1.6)	6 (1.8)	5 (1.4)		

AMT – antipsychotic monotherapy; APP – antipsychotic polypharmacy; AP – antipsychotic; FGAs – first generation antipsychotics; SGAs – second generation antipsychotics.

Table 5

Factors associated with adherence to the recommendations of Good Clinical Practice according to the univariate and multiple logistic regression analysis

Variables	Univariate logistic regression		Multiple logistic regression	
	OR (95% CI)	p	OR (95% CI)	p
Years of hospitalization (2014/15 vs. 2012/13)	1.58 (1.14–2.18)	0.006	1.53 (1.09–2.15)	0.015
Age of patients	0.97 (0.95–0.99)	0.001	0.96 (0.94–0.99)	0.005
Education of patients university and secondary vs. primarly	1.82 (1.18–2.81)	0.007	1.92 (1.23–3.02)	0.004
Duration of untreated psychosis up to 12 vs. over 12 mths	1.58 (1.14–2.19)	< 0.001		
Age of doctors up to 46 vs. older than 46 years	2.06 (1.48–2.88)	< 0.001	2.06 (1.45–2.93)	< 0.001
Experience as a specialist* up to 13 vs. more than 13 years	1.94 (1.40–2.69)	< 0.001		
Diagnosis	1.19 (1.04–1.36)	0.011		

***This variable highly correlated with the variable Age of doctors and was not included in the multivariate model; OR – odds ratio; CI – confidence interval.**

Discussion

In the last twenty years, the FPE treatment is in the focus in psychiatry research because of its influence on the course and outcomes of the disorder. The average age of the patients in this study was 28.5, most of them were males, with secondary education, unemployed, not married, and more than two-thirds lived with parents. Apart from the age, employment and living within the primary family, other findings were consistent with other studies [7, 14, 23]. A somewhat higher average age when diagnosing the first episode of schizophrenic disorder than most other studies, where patients were diagnosed for the first time in the mid 20s, can be explained by longer duration of DUP in the patients in this study [24, 25]. Also, this can be partly attributed to the fact that in the mentioned studies, the upper age limit of 30–40 years was set as the inclusion criterion. In other studies, with no upper age limit, and in the large national cohort studies with a similar operational definition of FPE, the average age is similar, or even significantly higher [5, 7, 23]. It is known that in the patients with schizophrenic disorders, the neurodevelopmental and neurocognitive changes are registered at the beginning of the disease and that a clear psychotic symptomatology usually preceded by a prolonged period with reduced functionality, which has a negative impact on the educational, social and professional achievements of the afflicted [26]. The educational structure and the great number of the unemployed and unmarried subjects are also a reflection of the situation in the society: according to the latest data from the Republic Institute for Statistics from 2016, less than one third of young people under 29 are employed, about 15% had elementary education, 60% secondary and about 25% (with a significant predominance of women) had a university degree, while only about 10% of them were married [27]. The great number of subjects living within the primary family can be partly explained by the socioeconomic factors, low employment rate and specific cultural patterns that significantly delay the separation of young people from parents in Serbia. More than one half of the subjects were taken to the psychiatric examination involuntarily by the police, while almost one quarter (23.7%) were hospitalized against their will. These findings can be explained by the specificity of the population of patients who were hospitalized at the institution in which the survey was conducted. Namely, the Clinic for Psychiatric Disorders „Dr Laza Lazarević“ is the only psychiatric institution in Belgrade (a city with more than 1.6 million inhabitants) where psychiatric emergencies are treated 24 hours a day, and the place where the involuntary examination and hospitalization are carried out. Therefore, it can be assumed that the proportion of agitated, auto and heteroaggressive, suicidal, partially and/or completed non-adherent patients and the patients with severe forms of illness in our sample was high. The frequency of involuntary hospitalization in the FPE varies widely from country to country, depending on the local specificities and legislative. Thus, the frequency of involuntary hospitalization in our sample was significantly higher than the average in Denmark and the United States, but significantly lower than in Canada [28–30]. The comparison data from Serbia were not available. The average duration of untreated psychosis of subjects in this study is 36.5 months and it was far above the average of about two years [31]. This finding is concerning, although it cannot be generalized due to the mentioned specificity of the sample. In the study that involved 263 patients hospitalized at the University Psychiatric Clinic in Belgrade for the first and second time, where the patients were treated exclusively voluntarily, the average duration of the disease was estimated at two years, regardless of the point at which the treatment started, which confirms our thesis [32].

Long duration of untreated psychosis has a negative impact on the treatment response, the course and outcomes of the disease, which is attributed to the maintenance of excitotoxicity in the untreated [33]. Poor outcomes include social consequences such as unemployment, homelessness, social isolation; also, the patients with a long DUP are at the increased risk of serious self-injury, suicide and heteroagression [34, 35]. The average of DUP varies across different countries, depending on the degree of development. In the developed countries, it is significantly lower in comparison to the underdeveloped and developing countries. That can be explained by a greater awareness of the disease, a more developed and accessible health service and the existence of intensive prevention and intervention programs in early psychosis [24, 36–39]. In the acute phase of the disease, the aims of the treatment are to establish control over disorganized or socially harmful behavior that endangers the safety of the patient and the environment, the mitigation of non-specific symptomatology (anxiety, agitation, insomnia), and the alleviation of positive psychotic symptoms. Ideally, all are achieved by the use of antipsychotic monotherapy which is a golden standard for the treatment of schizophrenia. The combination of antipsychotics is considered rational only in the absence of treatment response to monotherapy with FGAs, SGAs and clozapine. Since all antipsychotics (with the exception of clozapine) are considered to be practically equally effective, the choice of AP for initial treatment is primarily dependent on safety, i.e., profile of undesirable effects of the drug [16–19]. In spite of some dissonant tones, a consensus that there is no apparent difference between FGAs and SGAs in terms of efficacy, or in reducing the severity of positive psychotic symptoms is generally accepted. However, the patients treated with SGAs have lower rates of treatment discontinuation compared to FGAs, which is associated with a more frequent occurrence of extrapyramidal symptomatology and aggravation, or the induction of secondary negative and cognitive symptoms by FGAs [40]. The patients with the FPE also show certain specificities compared to those with chronic illness. Previously untreated patients often have a good response to therapy, the dose needed to achieve symptomatic remission is lower and the sensitivity to neurological and metabolic side effects of AP is greater [40, 41]. Therefore, most guidelines, including Serbian, for the people with the FPE recommend the mandatory administration of SGAs in lower and moderate doses in order to ensure the best adherence and prevention of relapses after stabilization of symptoms in the acute phase of treatment [16, 18, 19]. Four re-

cent cohort studies from the United States, Finland, Spain and the United Kingdom examined the patterns of prescribing AP in the FPE, so that they can serve for international comparisons [25, 42–44]. In our study, a total of 66.1% of subjects were treated with antipsychotic monotherapy with a total of ten antipsychotics (five from the class FGAs and SGAs). In 71.1% of patients treated with AMT, SGA is prescribed. Although there was no difference in the frequency of prescribing AMT before and after the introduction of a national guideline, a positive trend in the choice of AMT medication was observed. After 2013, the frequency of prescribing SGA as the AMT increased significantly compared to the previous period (78.41% vs. 63.5%). In the US and Finnish studies, the frequency of AMT and FGAs in the AMT was similar, about 90%, or about 9%, respectively [25, 42]. In the Spanish study in 2016, the frequency of prescribing AMT was slightly higher than our finding (69.2%), but the frequency of prescribing FGA as a monotherapy was only 3.7% [43]. In the British study from 2017, the frequency of prescribing AMT was 77.3%, with FGA accounting for 3.9% of prescriptions [44]. These differences in the findings could mean a better adherence to the recommendations of GCP in developed countries, which can be explained by the non-binding nature of our guide and the lack of regulatory bodies that would monitor the prescribing practice, which is mandatory in the Western countries [45].

On the other hand, the difference in the frequency of prescribing AMT could also be explained by the fact that the subjects in these studies (with the exception of Finland) were recruited from the community sample and they all signed an informed consent to the treatment and participation in the research. We are very likely to argue that they were more cooperative and in the longer remission that preceded the study period compared to our patients. Physicians have a professional obligation to regularly monitor new findings and treat the patients in accordance with the current professional rules and good clinical practice. Systematization of the latest scientific knowledge and gradations of their relevance to everyday medical practice through the development and implementation of official guidelines, GCP makes it easier for them to complete this task. In this study, it was estimated that in the prescribing of antipsychotics, there was an adherence to the recommendations of the GCP in 33.6% of subjects, with a significant increase in adherence to recommendations of GCP after 2013 (28.5% vs. 38.6%).

The factors independently associated with a compliance with the recommendations of GCP in this study were the year of hospitalization, the age of the prescriber as well as the age and education of the patient. In patients hospitalized after 2013, the GCP recommendations were adhered to 1.5 times more often than in patients treated in 2012 and 2013. This finding can be explained by the positive impact of the implementation of the National Guideline in the routine clinical practice of psychiatrists. With every year more of patient's age, the adherence to good clinical practice recommendations decreased by 4%. This finding can be explained in two ways. It is known that young, previously untreated patients have a strong initial response to therapy, so it is easier for doctors to comply with the basic principles of GCP for the treatment of FPE (SGAs antipsychotic monotherapy). On the other hand, it is possible that doctors are more attentive when treating young people, trying more to maintain adherence to the recommendations of GCP when choosing a medicine, to take more care of its safety and the long-term impact on physical health, cognition and overall functioning of patients who still have to accomplish themselves in their life roles. Perhaps this is also the case with the more educated patients, when the adherence to the recommendations was 1.9 higher than with those with the primary school. On the other hand, we cannot exclude possibility that the patients with the primary school education had lower premorbid achievements due to more pronounced neurocognitive impairments present before the appearance of the first clear psychotic symptoms, which would be in favor of more severe disease and a worse response to therapy. It is also possible that a better adherence of more educated patients with treatment facilitates the treatment process and allows for better adherence to the recommendations of the GCP.The factor most strongly associated with the adherence to the recommendations of the GCP was the age of a prescriber. Namely, those who were youger than 46 years of age complied with the recommendations of GCP twice more often than the ones over 46 years of age. It is possible that younger doctors were more motivated for continuous education, whereas older doctors may have ingrained habits and their favorite patterns of prescribing drugs and were not ready to try something new. On the other hand, it is possible that older, more experienced doctors treated more serious patients, when deviation from the recommendations of GCP is often inevitable.

Our study has some limitations. Firstly, due to the retrospective design of the study, we could not estimate objectively enough the differences in the severity of the disease in the patients. Also, we were not able to provide a clear insight into the reasons for prescribing certain antipsychotics, for switching antipsychotic therapy, or for prescribing antipsychotic polypharmacy. In addition, the research was conducted in only one, specific institution in Belgrade, which made it difficult to generalize the results.

Conclusion

After the introduction of the National Guideline of Good Clinical Practice for the Diagnosis and Treatment of Schizophrenia there have been significant, but insufficient changes in the prescribing patterns of antipsychotics during the treatment of the first psychotic episode in the daily clinical practice of psychiatrists in Serbia. There was an increase in prescribing of SGAs, which was used more often than FGAs, especially as the FGA. The use of FGA has decreased, but it has still been extremely common. One third of the patients with the FPE was treated with APF. Both of the individual characteristics of psychiatrists and patients have had a great influence on the prescribing patterns of antipsychotics. These data are important for the improvement of the rational use of drugs in the daily clinical practice of psychiatrists in Serbia.

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Received on October 02, 2017.
Revised on November 24, 2017.
Accepted on December 22, 2017.
Online First December, 2017.

ORIGINAL ARTICLE

UDC: 616.12
https://doi.org/10.2298/VSP171003010K

Implantable cardioverter defibrillator – powerful weapon in primary and secondary prevention of sudden cardiac death

Implantabilni kardioverter defibrilator – moćno oružje u primarnoj i sekundarnoj prevenciji iznenadne srčane smrti

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Abstract

Bacgraund/Aim. Sudden cardiac death (SCD) is one of the biggest problems of the contemporary medicine. Large studies showed that anti-arrhythmics, including amiodarone, are not effective in prevention of SCD in the patients with cardiac diseases who were on drug treatment. Those patients who received implantable cardioverter defibrillators (ICD) had better survival. The aim of this paper was to determine whether the patients receiving the ICD in the primary and secondary SCD prevention have longer survival than the patients treated exclusively with drug therapy. **Methods.** We included 1,260 patients with cardiac insufficiency and reduced left ventricular ejection fraction (LVEF < 35%) who were at high risk for malignant ventricular arrhythmias and SCD. Five hundred forty patients received ICD therapy. The cardiac resynchronization therapy – CRT/ICD group (n = 270) comprised the patients with cardiac insufficiency and CRT/ICD pacemaker at an optimal medical therapy. In the control group (n = 450), there were the patients with cardiac insufficiency (NYHA functional class 3–4, LVEF ≤ 35%, QRS duration ≥ 130 ms), at optimum drug therapy. **Results.** In the ICD group, there was a statistically significant increase in end-systolic volume (ESV) from 92.68 mL to 99.05 mL. In the group of patients with cardiac insufficiency who were on drug therapy, there was a significant decrease in LVEF (33.15% vs. 30.2%; $p = 0.017$), 6-minute walk distance (6 MWT distance) (216.55 m vs. 203.27 m, $p = 0.003$). In the same group, there was an increase in the values of ESV (90.19 mL vs. 95.41 mL; $p = 0.011$). An increase in the mortality rate in the group of patients with drug therapy compared to the CRT/ICD and ICD groups was statistically significant ($p < 0.05$). **Conclusions.** An ICD pacemaker implantation significantly reduces mortality compared to medical therapy only. In addition, the patients who have CRT in addition to ICD pacemaker, have a significantly better quality of life and increase in LVEF.

Key words:
death, sudden, cardiac; arrhythmias, cardiac; heart failure; defibrillators, implantable; drug therapy; pacemaker, artificial; mortality.

Apstrakt

Uvod/Cilj. Iznenadna srčana smrt (ISS) jedan je od najvećih problema savremene medicine. Velike studije pokazale su da antiaritmici, uključujući amiodaron, nisu efikasni u prevenciji ISS kod bolesnika sa srčanim oboljenjima koji su bili na medikamentnoj terapiji. Bolesnici kojima je ugrađen implantabilni kardioverter defibrilator (ICD) imali su bolje preživljavanje. Cilj ovog rada bio je da se utvrdi da li bolesnici kojima se ugrađuje ICD u primarnoj i sekundarnoj prevenciji ISS imaju duže preživljavanje u odnosu na one lečene isključivo lekovima. **Metode.** Uključili smo 1 260 bolesnika sa srčanom insuficijencijom i smanjenom ejekcionom frakcijom leve komore (LVEF < 35%) koji su imali povišeni rizik od maligne ventrikularne aritmije i ISS. Kod 540 bolesnika ugrađen je ICD pejsmejker. Terapija resinhronizacionim pejsmejkerom i ICD-om (CRT/ICD grupa) (n = 270) obuhvatila je bolesnike sa srčanom insuficijencijom i

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RT/ICD pejsmejkerom uz optimalnu medicinsku terapiju. U kontrolnoj grupi n = 450) bili su bolesnici sa srčanom insuficijencijom (NYHA funkcionalna klasa 3–4, LVEF ≤ 35%, QRS trajanje ≥ 130 ms, na optimalnoj terapiji lekovima). **Rezultati.** U ICD grupi postojalo je statistički značajno povecćanje ESV (end-sistolni volumen) od 92,68 mL do 99,05 mL. U grupi bolesnika sa srčanom insuficijencijom, koji su bili na terapiji lekovima, došlo je do značajnog smanjenja LVEF (33.15% vs. 30.2%; $p = 0{,}017$), distance nakon 6 minuta hodanja [6 MVT (216,55 m vs. 203,27 m; $p = 0{,}003$)]. U istoj grupi došlo je do povecanja vrednosti ESV (90,19 mL vs. 95,41 mL; $p = 0{,}011$). Povecćanje smrtnosti u grupi bolesnika sa terapijom samo lekovima u poređenju sa smrtnošću u CRT/ICD i ICD grupi bilo je statistički značajno ($p < 0.05$). **Zaključak.** Ugradnja ICD pejsmejkera značajno smanjuje smrtnost u poređenju sa lečenjem samo lekovima. Pored toga, bolesnici koji imaju CRT uz ICD pejsmejker, imaju znatno bolji kvalitet života i povecćanje LVEF.

Ključne reči:
smrt, iznenadna, srčana; srce, insuficijencija; defibrilacija srca; defibrilatori, implantabilni; lečenje lekovima; elektrostimulator srca; mortalitet.

Introduction

Sudden cardiac death (SCD) is one of the biggest problems of the contemporary medicine and its prevention is a challenge for every cardiologist. Most often, its occurrence presents the first and the last presentation of a cardiac disease. Many studies showed that the malignant arrhythmias are the main cause of the SCD. The secondary prevention of SCD is a treatment of the patients who survived cardiac arrest, or had the documented hemodynamically unstable ventricular arrhythmias. The primary prevention of SCD is a treatment of high-risk patients, but without documented previous cardiac arrest, or hemodynamically unstable ventricular arrhythmias. Ventricular tachycardia (VT) which degenerates in the ventricular fibrillation (VF) is the most often the cause of SCD. Practically, the effective management of VT is the prevention of SCD. Large multicentre studies showed that anti-arrhythmic, including amiodarone, are not effective in the prevention of SCD in the patients with cardiac diseases who were on drug treatment. Those patients who received implantable cardioverter defibrillators (ICD) had better survival.

Ventricular fibrillation and VT are the most common causes of SCD in the first 24 hours after an acute myocardial infarction (AMI). In the last 40 years, the in-hospital mortality had decreased from 30% to 10%–15% in AMI, mainly due to the prevention of a ventricular arrhythmias and conduction disorders. Ventricular fibrillation occurs most often during the first hour after AMI (80% of all cases occurs in the first 4 hours after AMI), and than its incidence rapidly decreases during 24–48 hours.

The epidemiological data show that the incidence of primary VF is significantly reduced, most probably, due to the correction of electrolyte disorders and other therapeutic measures that reduce the size of the myocardial infarction as well as due to the early use of beta-blockers. Unlike the primary VF caused by myocardial ischemia, which is the most frequent during the first hours after AMI, VF caused by a large necrosis, severe cardiac failure, left ventricular aneurysm and other severe AIM complications (secondary ventricular fibrillation) occurs later, after 48 hours, and has a poor prognosis [1].

Ventricular fibrillation and VT can only be stopped by the use of DC shock or defibrillators, and it is necessary to continuously apply the reanimation measures to maintain the vital functions, until its application. The occurrence of these vital threatening rhythm disorders does not depend on the size of the myocardial necrosis, and even very small AMI that otherwise have a good prognosis, could lead to the SCD.

Implantable cardioverter defibrillators (ICD) are devices that are designed primarily to perform the therapy of life threatening rhythm disorders. Today, the modern ICD devices have similar design and function as the standard bradycardic pacemakers. They use a lithium-vanadium battery due to the reliability of the energy source and the need to deliver a higher amount of energy in a short period of time.

Detection of malignant ventricular arrhythmias is a specific and basic function of an ICD. The biggest advance in technology of those devices was the introduction of a gradual "tiered" therapy. This implies that the detected VT is treated with the least aggressive therapy applied through the antitachycardia burst stimulation with the different duration of the V-V stimulus. Contemporary devices use synchronous cardioversion with a low electrical power of a DC shock and finally defibrillation with the maximum DC shock strength current (30–40 J) in the case of VF [2, 3].

Several studies demonstrated the superiority of an ICD in comparison to a drug therapy in the primary SCD prevention. The MADIT study showed 54% reduction in overall mortality in the patients with a left ventricle ejection fraction (LVEF) < 35% who received ICD due to ischemic heart disease. In the MUSTT study, the patients with ICD and LVEF < 35% had a lower mortality rate due to arrhythmia by 75% and a reduction in overall mortality of 60%. The SCD-HEFT study showed that the patients with the congestive heart failure in New York Heart Academy (NYHA) class II and III, with LVEF<35% on the optimal drug therapy, who ICD, had mortality decreased by 23%. The MADIT II study examined the benefit of ICD implantation in the patients with coronary artery disease who had LVEF < 30% and at least one AMI. An absolute reduction in mortality rate by 31% was observed in the group of patients with ICD compared to those with conventional therapy [4, 5].

The largest studies which examined the efficacy of an ICD in a secondary SCD prevention were: AVID, CIDS, CASH and DUTCH CES. In each of the mentioned study, a significant and undoubtedly improved survival was registered in the patients with ICD compared to those who received anti-arrhythmic drugs (reduction of 20% in mortality

rate in the CIDS study, 38% and 39% in the CASH and AVID studies and of 73% in the DUTCH CES study) [6, 7].

The aim of this paper was to determine whether the patients receiving ICD in the primary and secondary SCD prevention have longer survival than the patients treated exclusively with drug therapy.

Methods

The study included the patients who were treated at the Clinic for Cardiovascular Diseases, Clinical Center Niš from 2007 to 2016 due to heart failure symptoms. The study was conducted according to the human rights and ethical principles for medical research from the Declaration of Helsinki, World Health Organization (WHO).

We included 1,260 patients with cardiac insufficiency and reduced ejection fraction (LVEF < 35%) who were at high risk for malignant ventricular arrhythmias and SCD.

Within the study, 540 patients received ICD therapy (ICD group) based on ICD compliance criteria. We included the patients with left ventricular dysfunction, at least 40 days after a large AMI infarction, and with LVEF ≤ 35% for the primary SCD prevention. For secondary SCD prevention, we included the patients who survived VF or haemodynamically unstable VT, who had non-ischemic dilated cardiomyopathy and a significant left ventricular dysfunction and life expectancy of at least a year. The cardiac resynchronization therapy (CRT/ICD) group (n = 270) comprised the patients with the cardiac insufficiency and cardiac resynchronization therapy CRT/ICD pacemaker (NYHA functional class III-IV, LVEF ≤ 35%, QRS duration ≥ 130 ms, dilated left ventricle (LV > 55 m), at optimal medical therapy for cardiac insufficiency with the fulfilled echocardiographic criteria for responsiveness to the CRT [pre-ejection period of LV (PEPLV) > 140 ms, difference between PEP LV and PEP RV period > 40 ms, SPWMD (septal to posterior wall motion delay > 135 ms). In the control group (n = 450), there were the patients with cardiac insufficiency (NYHA functional class III-IV, LVEF ≤ 35%, QRS duration ≥ 130 ms, at optimum drug therapy) who did not meet the criteria for ICD therapy as well as for CRT.

Patients in the control group were only on optimal drug therapy that included β blocker, ACE inhibitor, diuretic, digitalis and anti-arrhythmic amiodarone (if refused to receive ICD in primary prevention). In all patients, we performed 12 channel electrocardiography (ECG), echocardiographic examination, and six-minute walking test (6MWT), examination of signs and symptoms of heart failure and drugs that were used. After an average follow-up period of one year from ICD or CRT/ICD implantation, ECG, echocardiographic examination, 6MWT distance, signs and symptoms of heart failure were compared together with the number of hospitalizations due to heart failure.

The procedures of pace-maker implantation were performed in sterile conditions, in the cardiac catheterisation laboratory using the Simens Axiom Artis fluoroscopy apparatus. The Pacemaker System Analyzer (PSA) (Figure 1) was also an integral part of the instrumentation used in implantation along with the sterile cables for the connection between the sterile pacemaker and non-sterile PSA. During the implantation, a sterile set of surgical instruments on a sterile stand was used (Figure 2).

Fig. 1 – Pacemaker programmer.

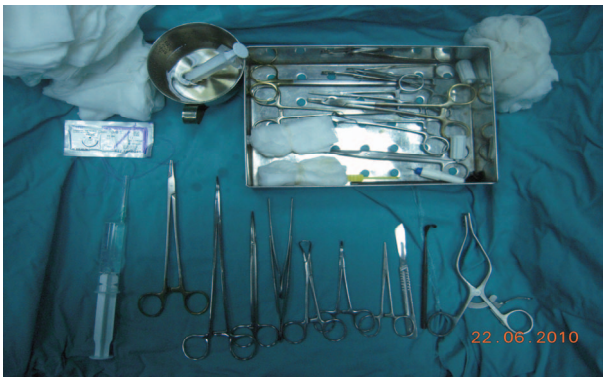

Fig. 2 – Surgical instruments for pacemaker implantation.

ICD pace-maker used for primary prevention was programmed for detection and therapy in the VF zone only (for heart rate mostly > 207/min). In the secondary prevention, in addition to the VF zone for detection and therapy, the VT zone was also activated. That included anti-tachycardia pacing (ATP) therapy options, and in case of its failure to interrupt the arrhythmia, an option to activate the DC shock.

Methods of descriptive and analytical statistics were used in the processing of the data obtained from the research. The selection of methodological procedures was adjusted to the aims of the study and to scientific hypotheses. All obtained parameters were statistically processed by the percentage ratio, mean values of numerical features, standard deviation, Student's *t*-test and χ^2 test, survival rate according to different therapy of cardiac insufficiency (Kaplan Meier curve), Cox regression model – risk factors for fatal outcome.

Results

The technical parameters obtained during the pacemaker implantation are given in Table 1. The gender and age structures of the examined groups are given in Table 2. By analyzing the parameters in Table 3, in the group of patients with CRT/ICD, we observed statistically significantly lower values of all parameters after the implantation of device compared to the same parameters before the implantation, (QRS 147.33 ms vs. 124.44 ms; LVEF 25.21% vs. 37.63%;

6-MWT distance 224.45 m vs. 289.9 m; EDV 286.89 mL vs. 181.22 mL; ESV 187.7 mL vs. 110.5 mL; PEP LV – 181.44 ms vs. 147.18 ms, PEP RV 114.3 ms vs. 96.21 ms; SPWMD 194.5 ms vs. 139.48 ms), $p < 0.001$. In the group of patients with ICD, there was a statistically significant increase in ESV (end-systolic volume) from 92.68 mL to 99.05 mL ($p < 0.001$), as well as an increase in PEP LV from 124.89 ms to 129.95 ms ($p = 0.003$). In the group of patients with cardiac insufficiency who were on drug therapy, there was a significant decrease in LVEF (33.15% vs. 30.2%; $p = 0.017$), 6 MWT distance (216.55 m vs. 203.27 m; $p = 0.003$). In the same group, there was an increase in the values of ESV (90.19 mL vs. 95.41 mL; $p = 0.011$) as well as in PEP LV parameters (122.75 ms vs. 128.56 ms; $p = 0.034$).

Table 1

Parameters obtained during pace-maker implantation

Parameters	CRT-D (n = 270)	ICD (n = 540)
Pacing treshold A (volt, 0.5 ms), mean ± SD	1.17 ± 0.77	0.8 ± 0.47
Pacing treshold RV (volt, 0.5 ms), mean ± SD	0.67 ± 0.69	0. 7 ± 0.4
Pacing treshold LV (volt, 0.5 ms), mean ± SD	1.85 ± 0.8	–
Sensing A, mean ± SD	2 ± 0.75	1.8 ± 0.65
Sensing RV, mean ± SD	8 ± 3.8	11 ± 2.8
Sensing LV, mean ± SD	12 ± 4.6	–
Procedure duration (min), mean ± SD	91 ± 24.8	32 ± 17.6
Radiation exposure duration (min), mean ± SD	7.7 ± 4.9	2.1 ± 0.3
Received radiation dose (μGy/m^2), mean ± SD	1756 ± 321.1	217 ± 93
Complications (n)		
haemathoma	25	19
pneumothorax	0	0
infection	0	0
coronary sinus perforation	0	–
extracardiac stimulation	6	19

CRT-D – cardiac resynchronization therapy defibrillator; ICD – imeplantable cardioverter defibrillator; A – atrium; RV – right ventricle; LV – left ventricle; SD – standard deviation.

Table 2

Gender and age structure of the study groups

Parameters	Group		
	ICD (n = 540)	CRT/ICD (n = 270)	Drug therapy (n = 450)
Sex, n (%)			
male	386 (71.5)	224 (83.0)	350 (77.8)
famele	154 (28.5)	46 (17.0)	100 (22.2)
Age (year)			
mean	62.43	57.98	63.44
SD	8.93	14.35	6.96
min	33	23	44
max	75	73	76

For abbreviation see under Table 1.

Table 3

Investigated parameters at the beginning of the study and after follow-up period

Parameters	CRT/ICD		ICD		Drug therapy	
	Before mean ± SD	After mean ± SD	Before mean ± SD	After mean ± SD	Before mean ± SD	After mean ± SD
QRS (ms)	147.33(10.30)	124.44(10.66)*	113.16(5.58)	113.95(5.91)^{ns}	103.86(9.37)	104.32(8.90)^{ns}
LVEF (%)	25.21(5.08)	37.63(8.37)*	27.16(6.59)	27.00(5.89)^{ns}	33.15(6.26)	30.21(5.75)‡
6 MVT (m)	224.45(38.53)	289.90(67.63)*	209.89(28.18)	213.11(32.62)^{ns}	216.55(28.73)	203.27(30.22)†
EDV (mL)	286.89(55.81)	181.22(44.38)*	166.37(24.40)	164.11(23.97)^{ns}	156.36(33.13)	157.45(34.03)^{ns}
ESV (mL)	187.70(50.63)	110.50(22.33)*	92.68(21.19)	99.05(21.43)*	90.19(14.61)	95.41(18.67)‡
PEP LV (ms)	181.44(17.58)	147.18(8.57)*	124.89(6.93)	129.95(5.13)†	122.75(9.87)	128.56(6.13)‡
PEP RV (ms)	114.30(20.41)	96.21(17.31)*	110.68(13.76)	111.58(12.79)^{ns}	110.09(13.77)	111.50(12.39)^{ns}

EDV – end-diastolic volume; ESV – end-systolic volume; PEP – pre-ejection period; LV – left ventricle; RV – right ventricle; LVEF – left ventricle ejection fraction; GVT – six-minutes walking test; SPWDM – systolic delay of the posterior wall.
***$p < 0.001$; $^{†}p < 0.01$; $^{‡}p < 0.05$; ns – non significant.**
For other abbreviatons see under Table 1.

In the group of patients with resynchronization therapy before CRT/ICD pacemaker implantation, 207 (76.6%) patients had NYHA functional class III and 54 (20.1%) patients had NYHA functional class IV (Table 4). After the implantation of abovementioned device, 18 patients had NYHA class 4, 72 (26.6%) patients were in NYHA 2 class, and 180 (66.6%) patients had NYHA class III of heart failure.

In the group of patients before ICD was implanted, 225 (41.6%) had NYHA II functional class, and after its implantation 270 (50%) patients were in NYHA class II. In the group of patients with drug therapy, 171 (38%) patients were in the NYHA class II, and after the follow-up period, 153 (34%) patients were in the same functional class. After the follow-up period, in the group of patients treated with medicaments, the percentage of those with NYHA class IV increased from 2% to 6%.

In the group of patients with ICD, 27 patients, or 5% died after the follow-up period of at least a year (Table 5). In the group of patients with CRT/ICD device, 18 patients, or 6.7% had a fatal outcome, while in the drug therapy group, this number was 108, or 24% deaths during the follow-up. An increase in a mortality rate in the group of patients with drug therapy compared to the CRT/ICD and ICD groups was statistically significant ($p < 0.05$). There was no significant difference in mortality between the ICD and CRT/ICD groups.

In the group of patients with CRT/ICD, 72 (26.6%) patients had the pacemaker activation/switch on [anti-tachycardia pacing (ATP) or DC shock], while in the ICD group, 21 (35%) patients had the pace-maker activation.

Observing the length of survival in the analyzed groups, it was detected that the patients in the CRT/ICD group had the longest survival [391.7 days with statistical error (SE) = 7.913 days], statistically significantly longer than the subjects in the group of patients with the drug therapy, whose survival was 329.892 ± SE = 10.11 days (Log Rank χ^2 = 9.728; $p < 0.01$) (Figure 3). The patients with implanted ICD had an average survival of 351.6 days with SE = 9.01 and also a significantly longer survival than the patients with the drug therapy (Log Rank $\chi^2 = 7.623$; $p < 0.05$).

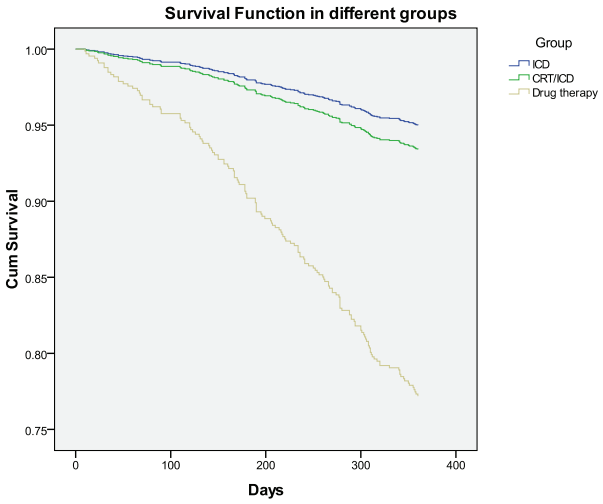

Fig. 3 – Kaplan-Meier curves in the study groups. For abbreviations see under Table 1.

Sudden cardiac death is defined by the WHO as a death related to any cardiac disease that occurs outside the hospital, in an emergency department, or immediately upon arrival to the hospital, or within an hour. It is thought that in the USA, for over 45% of all patients who dies of cardiac disease, the immediate reason is SCD [8–10]. The greatest number of SCD is caused by arrhythmias.

Table 4

NYHA class at the beginning and at the end of study

NYHA class	ICD n (%)	CRT/ICD n (%)	Drug therapy n (%)
Before			
II	225 (41.6)	9 (3.3)	171 (38)
III	225 (41.6	207 (76.6)	270 (60)
IV	90 (16.8)	54 (20.1)	9 (2)
After			
II	270 (50.0)	72 (26.6)	153 (34)
III	225 (41.6)	180 (66.6)	270 (60)
IV	45 (8.4)	18 (6.8)	27 (6)

NYHA – New York Heart Association.
For other abbreviations see under Table 1.

Table 5

Mortality in the study groups

Mortality	CRT/ICD n (%)	ICD n (%)	Drug therapy n (%)	Total n (%)
No	252 (93.3)	513 (95)	342 (76)	999 (79.2)
Yes	18 (6.7)	27 (5)	108 (24)*	261 (20.8)
Total	270 (100.0)	540 (100.0)	450 (100.0)	1,260 (100.0)

For abbreviations see under Table 1.
***$p < 0.05$ (vs. CRT/ICD and ICD groups).**

Discussion

Among them, the cause are the ventricular tachycardia and fibrillation in over 80%, and for the rest of patients bradyarrhythmias and asystole. The coronary artery disease is responsible for arrhythmias in at least 80% of these patients, and dilated idiopathic cardiomyopathy and hypertrophic cardiomyopathy are the next most common cause. A survival after cardiac arrest varies from less than 5% to 60% depending on the characteristics of the cardiac arrest (e.g., cardiac ethiology or not, the presence of other people or not at that moment, VF or not) [11–14]. The results of cardiopulmonary resuscitation (CPR) do not depend only on the quality of the reanimation, but also on the patient's condition before the start of CPR. It is now generally accepted that the time of electrical defibrillation is the most important determinant of survival after a cardiac arrest. The introduction of automatic external defibrillators is allowed for use by even less-trained users to deliver electric shock in cases of out-of-hospital VF or VT, often several minutes before the arrival of the medical emergency medical team. The strategy is also known as the "first defibrillator response" [15–17].

Prevalence of cardiac insufficiency is an increasing problem in modern cardiology. Cardiac insufficiency as a diagnosis is in the expansion and more and more patients have this diagnosis. In our study, there were 1,290 patients with this diagnosis. There were no statistically significant differences regarding the gender and age between investigated groups. Sudden cardiac death is the most common cause of death in the patients with cardiac insufficiency. The patients with NYHA class II have 1-year mortality of 5%–15%, while the patients with NYHA class III have an annual mortality of 20%–50%, and the most vulnerable patients in NYHA class IV have an annual mortality of 30%–70% [16–18]. The patients with a higher NYHA class die of cardiac insufficiency and its repercussions on other organs while the patients with a lower NYHA class die more often from SCD. In the group of patients with CRT/ICD device, 76.6% had NYHA class III, and 20.1% had NYHA class IV. After the implantation of CRT/ICD pacemaker, 26.6% of patients were in NYHA class II and 66.6% in NYHA class III [15, 17, 19].

In the group of patients with CRT/ICD, the percentage of patients with NYHA functional class II had increased from 41.6% to 50% after the follow-up period. In the group with the drug therapy, the percentage of patients with NYHA class II decreased during the follow-up from 38% to 34%, but the percentage of patients with NYHA functional class IV increased from 2% to 6%.

Dilated cardiomyopathy is frequently the final result of ischemic heart disease. In our study, the highest percentage of patients had an ischemic heart disease as the cause of dilated cardiomyopathy, and the rest of patients had idiopathic dilated cardiomyopathy. Implantation of an ICD pacemaker is the only effective therapy for SCD prevention. It is known that the highest risk of SCD have patients with previous cardiac arrest, sustained VT, family history of SCD, extreme left ventricular hypertrophy, etc. Also, presyncope in the anamnesis could represent a strong predictor of high risk of SCD [18–20].

In our research, there was also a group of patients who survived VT or VF, and found no organic substrate on the myocardium, probably because we were unable to perform an endomyocardial biopsy. Therefore, we were not able to clarify the cause of arrhythmias in these patients.

The MADIT study showed a 54% reduction in the overall mortality in the patients with an LVEF < 35% and ICD implanted, due to ischemic heart disease. The MUSTT study in the patients with LVEF < 35% showed that the reduction in mortality due to arrhythmia in the patients who received ICD pacemaker was 75%, and a reduction in the overall mortality was 60%. These two studies were the first which investigated ICD pacemaker implantation for primary prevention in the high-risk patients. They showed that the left ventricle dysfunction is the strongest predictor of SCD. The decrease in LVEF after AMI by 5% increased the risk of SCD by 21% in the first month. The SCD-HEFT study was designed to show whether amiodarone, or ICD reduce total mortality in the patients with coronary artery disease, or non-ischemic cardiomyopathy, classified as NYHA class II or III, and those who had LVEF less than 35%. The patients were randomized to 3 cohorts: 847 with placebo, 845 with amiodarone, and 829 with ICD. The main conclusion of the study was that the patients with a congestive heart failure in NYHA class II or III, with LVEF < 35%, with an optimal drug therapy, had a mortality in the controlled placebo group of 7.2% per year for 5 years. A simple ICD, programmed only for the detection zone of 188/min, and for therapy only with the maximal DC shock, reduces mortality by 23%. Amiodarone, when taken as a primary prevention does not increase survival and it has the same effect as placebo in the primary prevention. The MADIT II study examined the prophylactic benefit of ICD in the patients with coronary artery disease, LVEF < 30%, who had at least one myocardial infarction [4–7]. The patients with ICD had 31% of mortality reduction compared to the group of patients with the conventional therapy. Both groups of patients had equivalent and necessary doses of beta-blockers, ACE inhibitors, diuretics, digitalis and aspirin. The conclusions of the MADIT II study showed that in patients with a previous myocardial infarction and left ventricular dysfunction, the prophylactic ICD pacemaker significantly increased survival.

The patients with the greatest benefit of ICD implantation are those who are prone to malignant arrhythmias: congenital heart disease, hypertrophic cardiomyopathy, Brugada syndrome, idiopathic VT/VF, left ventricular noncompaction cardiomyopathy, long and short QT syndromes, arrhythmogenic right ventricular dysplasia, infiltrative cardiomyopathy [4, 5].

Secondary prevention is indicated in the patients who survived a cardiac arrest or sustained VT. Numerous randomized studies showed that the use of ICD is associated with a reduction in mortality compared to the patients treated with any type of drug therapy. The most important of these are the AVID, CIDS, and CASH studies. In each of the ICD studies, significant and undoubtedly improved the survival compared to anti-arrhythmic drugs was demonstrated (from 20% in the CIDS study, more than 38% and 39% in the CASH and AVID studies to as much as 73% in the Dutch DUTCH CES study). In our study we found a statistically significant difference in survival of patients with primary and secondary prevention (received ICD pace-

maker) compared to the patients who were on the drug therapy (24% vs. 5%, respectively) [6, 7]. Also, the patients who had resynchronization therapy (CRT pacemaker implanted) together with ICD had better quality of life, a statistically significant increase in LVEF compared to other groups of patients (36.27% vs. 24.63%, respectively), as well as the improvement of other echocardiographic parameters.

Conclusion

Based on the results of this study, it can be concluded that ICD pacemaker implantation significantly reduces mortality, both in primary and secondary prevention, compared to medical therapy only. In addition, the patients who have CRT in addition to ICD pacemaker, have a significantly better quality of life, an increase in LVEF and the echocardiographic parameters which are indices of left ventricular function. Also, the use of medication therapy in the patients with the implanted pacemakers reduces the frequency of DC shock delivery which preserves the myocardial function and reduces the damage that occurs during electrical current passes through the heart muscle. In the end, it was shown that the patients who have received the resynchronization therapy have the highest benefit from the implantation of the pacemaker system in terms of survival.

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Received on October 3, 2017.
Accepted on January 9, 2018.
Online First January, 2018.

ORIGINAL ARTICLE

UDC: 612.017:616.61
https://doi.org/10.2298/VSP170714008O

Successful treatment of idiopathic retroperitoneal fibrosis with combined immunosuppressive therapy

Uspešno lečenje idiopatske retroperitonealne fibroze kombinovanom imunosupresivnom terapijom

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Abstract

Background/Aim. Idiopathic retroperitoneal fibrosis (IRF) is characterized by the fibroinflammatory periaortic tissue that affects the ureters, causing obstructive nephropathy and variable impairment of renal function. The findings strongly suggest an autoimmune etiology. The optimal treatment has not been established. The aim of this study was to analyze a long-term efficacy of combined corticosteroid therapy with mycophenolate mofetil (MMF) in the patients with IRF. **Methods.** We retrospectively followed 13 patients (8 males and 5 females) with IRF. All patients received corticosteroids and MMF. For the patients with severe renal failure, an initial ureteral decompression was made and prednisone was started orally 0.5 mg/kg with fast tapering. In cases with a mild renal failure corticosteroids were administrated as intravenous methylprednisolone pulses for 3 days, followed by oral prednisone. The dose of MMF was 1000 mg twice a day. MMF was stopped after 18 months and prednisone after 48 months. **Results.** Systemic symptoms resolved in all patients. Erythrocyte sedimentation (SE) rate declined from the mean of 67.6 to 26.3 mm/h and C-reactive protein (CRP) from the mean of 18.5 to 6.3 mg/L. In 7 out of 8 patients, the ureteral stents were successfully removed 13 weeks on average. Seven patients had 100% of reduction in the periaortic mass, and the average percent reduction was 76.9%. The kidney function improved and remained normal in 6 treated patients. In 4 patients a mild chronic renal failure remained due to afunction of one kidney. Three patients, with a prior chronic renal failure, did not get worse renal function. The disease recurred in 3 patients. There were no treatment side effects noted. **Conclusion.** Combination of corticosteroids and MMF is a potentially effective treatment in restoring the renal function and reducing the fibrotic tissue in the patients with idiopathic retroperitoneal fibrosis. It could prevent the need for ureteral stenting and surgery. Longer treatment may reduce a possibility of recurrence.

Key words:
retroperitoneal fibrosis; renal insufficiency; autoimmune diseases; adrenal cortex hormones; mycophenolic acid; remission induction; recurrence.

Apstrakt

Uvod/Cilj. Idiopatska retroperitonealna fibroza (IRF) karakteriše se periaortnim fibroinflamatornim tkivom koje zahvata uretere dovodeći do opstruktivne nefropatije i različitog stepena bubrežne insuficijencije. Oboljenje je najverovatnije autoimune etiologije. Optimalna terapija do sada nije definisana. Cilj rada bio je analiza dugoročne efikasnosti kombinovane imunosupresivne terapije kortikosteroidima i mikofenolat mofetilom u lečenju IRF. **Metode.** Retrospektivno je praćeno 13 bolesnika (8 muškaraca i 5 žena) sa IRF. Svi bolesnici su primili kortikosteroide i mikofenolat mofetil (MMF). Kod bolesnika sa izraženom bubrežnom insuficijencijom, prvo je učinjena dekompresija urinarnog trakta i potom započeta terapija peroralnim prednizonom 0,5 mg/kg sa brzim smanjivanjem doze. Bolesnici sa umerenom bubrežnom slabošću primili su inicijalno tri pulsne doze metilprednizolona intravenski, a potom je nastavljeno sa prednizonom. Doza MMF je bila 1000 mg dva puta dnevno. MMF je obustavljen nakon 18 meseci, a prednizon nakon 48 meseci. **Rezultati.** Kod svih bolesnika došlo je do povlačenja opštih simptoma bolesti. Vrednost sedimentacije eri-

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trocita (SE) smanjena je sa prosečnih 67,6 na 26,3 mm/h, a C-reaktivnog proteina (CRP) sa prosečih 18,5 na 6,3 mg/L. Kod sedam od ukupno osam bolesnika, ureteralni stentovi su uspešno izvađeni nakon prosečno 13 nedelja. Sedam bolesnika imalo je 100% redukciju periaortnog tkiva, a prosečni stepen redukcije je bio 76,9%. Bubrežna funkcija je poboljšana, kod šest bolesnika je normalizovana, dok je kod četiri zaostala umerena hronična bubrežna slabost usled afunkcije jednog bubrega. Tri bolesnika sa prethodnom hroničnom bubrežnom insuficijencijom nisu pogoršala funkciju. Recidiv bolesti imala su tri bolesnika. Nisu registrovani neželjeni efekti terapije. **Zaključak.** Kombinovana primena kortikosteroida i MMF je potencijalno efikasna terapija u poboljšanju bubrežne funkcije i smanjenju debljine fibroznog tkiva kod bolesnika sa IRF. Ona bi mogla otkloniti potrebu za plasiranjem ureteralnih stentova i hirurškim lečenjem. Duže trajanje terapije moglo bi smanjiti mogućnost recidiva bolesti.

Ključne reči:
retroperitonealna fibroza; bubreg, insuficijencija; autoimunske bolesti; kortikosteroidni hormoni; mikofenolna kiselina; remisija, indukcija; recidiv.

Introduction

Retroperitoneal fibrosis (RF) is characterized by the development of inflammatory fibrotic tissue surrounding the infrarenal aorta, the iliac arteries and other retroperitoneal structures. The fibrotic tissues spread laterally and entrap one or both ureters causing obstructive uropathy and variable impairment of renal function. About one-third of all cases of RF are secondary to certain drugs, malignant disease, infection, radiation therapy or surgery. The remaining two-third of RF cases are considered idiopathic as no specific cause can be identified [1].

Idiopathic retroperitoneal fibrosis (IRF) is a rare disorder with an estimated annual incidence of 0.1–1.3 cases per 100,000 persons [2]. Males are affected twice to three times more frequently than females with the mean age at presentation between 50 and 60 years, but it has also been reported in children and other adults [1, 3].

The pathogenesis is not clear. Parum et al. [4] postulated that the disease could be the result of an inflammatory state triggered by an autoimmune response to some antigens in atherosclerotic plaques of the abdominal aorta. Recent findings suggest a systemic nature of the disease with the presence of constitutional symptoms, elevated acute phase reactants, often positive autoantibodies (especially antinuclear antibodies) and concomitance of the other autoimmune conditions [1, 5]. The current researches consider IRF as part of the IgG4-related disease, but this association was proved only in 30%–60% of cases [6, 7].

The clinical presentation of idiopathic retroperitoneal fibrosis (IRF) is often insidious with the localized symptoms due to the compressive effects of the retroperitoneal mass (abdominal or back pain, leg oedema, oligoanuria and uraemia), and the systemic symptoms (fatigue, fever, anorexia, weight loss) due to the inflammatory nature of the disease [1].

The diagnosis is usually made by either computed tomography (CT) or magnetic resonance imaging (MRI) of abdomen. These techniques can visualize the extent of the fibrosis and determine the possible presence of the tumour or lymphadenopathy. A confirmatory biopsy is sometimes needed (atypical localisation, therapy nonresponding cases) [8].

Considering the possible autoimmune etiology, various immunosuppressive (IS) drugs were successfully used in the nonsurgical management of IRF [1, 9–13]. However, the experiences in treatment are mainly based on the observations of case reports, or small groups of patients. So far, the optimal IS agent, the dose and the length of the treatment have not been established [1].

The aim of this retrospective study was to analyze a long-term efficacy of combined steroid therapy with mycophenolate mofetil (MMF) in the patients with IRF.

Methods

Patient population

From January 2004 to May 2016, 13 patients were referred to the Nephrology Clinic for management of IRF. All patients underwent the CT or MRI, intravenous urography and ultrasound examination (US).

The patients were asked about past or current use of methysergide, β blockers, ergotamine, methyldopa, or a history of recent infections, abdominal trauma, pelvic or abdominal surgery and external beam radiation. They, all underwent the appropriate cancer screening, according to the gender and age. The IRF diagnose was based on characteristic clinical and CT findings. Two patients had prior histological confirmation from the biopsy material taken during ureterolysis.

The baseline laboratory screening included the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood count, chemistry profile with creatinine, antithyroid peroxidase antibodies (anti-TPO antibodies), thyroglobulin antibodies, thyroid stimulating hormone (TSH), testing for antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA) and rheumatoid factor (RF).

Treatment

All patients received steroids with MMF. The patients with severe acute renal failure received a double-J ureteral stent (DJS) or percutaneous nephrostomy (PNS). After that, steroids were started as oral prednisone 0.5 mg/kg/day for one month, then tapered to maintenance of 10–5 mg/day. In the patients with a mild renal failure with no placement of DJS/PNS, steroids were given as an intravenous methylprednisolone pulses: 250 mg/day each for three consecutive days, followed by oral prednisone 0.5 mg/kg/day for one month, with tapering the dose as mention above. MMF was administered orally in a dose of 1000 mg twice daily for the first 6

months, then reduced to 500–750 mg twice daily, as the maintenance dose, till the end of 18 months. Steroids were stopped after 48 months from the start of the therapy.

Follow-up

All patients were followed monthly in the first 6 months, then every 3 months till the end of the second year. After that, the patients were seen once in 4–6 months. At each control, the patients were submitted to the clinical examination, US examination and to the following laboratory tests: ESR, CRP, serum creatinine level, complete blood count and urine analysis.

Normal range for ESR according to gender was: 0–25 mm/h in males, 0–30 in the females, and for CRP 0–5 mg/L.

The CT scan, or MRI of abdomen was performed at 6, 12, 24 and 48 months after the initiation of the therapy. After that, the CT scan, or MRI was performed in the case of suspected recurrence of disease.

A decision to remove the ureteral stents, or PNS was made in collaboration with an urologist. It was based on the improvement in laboratory parameters and radiographic evidence that the fibrotic mass no longer encased the affected ureter.

Active disease was defined by the presence of a periaortic mass surrounding one of both ureters with hydronephrosis at CT/MRI associated with an increase in CRP and/or ESR.

Remission of the disease was defined by a regression of hydronephrosis and by a reduction of the fibrotic tissue at CT/MRI in comparison with the basal examination together with the normalization of CRP and/or ESR.

Recurrence of the disease was defined by the CT/MRI-proven increase of the periaortic mass with, or without entrapment of one, or both ureters associated with a new increase in CRP and/or ESR.

Radiographic review

The abdominal cross sectional imaging either by the contrast enhanced CT, or MRI was reviewed by a single radiologist. The patients were classified based on the extent of the soft-tissue mass verified on the first visit using a classification previously described by Scheel and Feeley [14]: class I: the soft-tissue density surrounding the infrarenal aorta and/or iliac vessels; class II: the soft-tissue density surrounding the infrarenal vena cava; class III: the lateral extension of inflammation/fibrosis with compression of one or both ureters; class IV: the extension of fibrosis to include the renal hilum with the compression of the renal artery and/or renal vein.

The patients could be categorized in multiple classes based on the extent of disease seen on imaging. The temporal change in the disease was determined by measuring the thickness of soft tissue relative to the aorta on the CT scan, or MRI.

Statistical analysis

The complete statistical analysis of data was done using the statistical software package, PASW Statistics 18® [SPSS (Hong Kong) Ltd., Hong Kong]. All variables were presented as frequency of certain categories. The χ^2 test was used for analysing the significance of differences of categorical variables. The continuous variables were presented as means and standard deviations, or median with a range and were compared using the Mann-Whitney *U* test, or the Kruskal-Wallis test. The distribution normality was tested by using the Shapiro-Wilk test (number of subjects was less than 50). All analyses were estimated at $p < 0.05$ level of the statistical significance.

The principles of International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice guidelines were strictly followed. Ethical approval from the Ethics Committee of the Military Medical Academy was obtained for the study protocol on January 21st, 2016.

Results

Of the 13 patients, there were 8 males and 5 females (Table 1). The mean age at the time of diagnosis was 54.0 ± 6.9 years (range 36–60 years). The patients were followed for a mean period of 99.1 ± 34.6 months (median 99.4, range 41.6–150.1 months).

Table 1

Demographic and clinical characteristics in the patients with retroperitoneal fibrosis

Patient characteristics	Values
Age (year), mean ± SD (range)	54 ± 6.9 (36–60)
Male, number (%)	8 (61.5)
Female, number (%)	5 (38.5)
First presentation/recurrence, number	10/3
Symptoms on presentation, cumulative number (%)	
weight loss	11 (84.6)
back pain	8 (61.5)
fatigue	7 (53.8)
nausea	4 (30.8)
leg edema	4 (30.8)
abdominal pain	3 (23.1)
new onset of hypertension	2 (15.4)
both (abdominal, back) pain	1 (7.7)
hydrocele	1 (7.7)

SD – standard deviation.

Table 2

Baseline and follow-up laboratory parameters

Parameters	Baseline mean ± SD	Follow-up mean ± SD	*p* value*
ESR (mm/hr)	67.6 ± 33.8	26.3 ± 30.2	$p < 0.001$
CRP (mg/L)	18.5 ± 10.4	6.3 ± 5.2	$p < 0.001$
WBC ($\times 10^9$/L)	8.3 ± 1.7	8.6 ± 1.6	$p = 0.001$
Hgb (g/L)	113.7 ± 18.1	141.1 ± 17.2	$p < 0.001$
Serum creatinine (μmol/L)	334.5 ± 326.3	124.5 ± 69	$p = 0.010$
GFR (mL/min)	33.3 ± 21.6	59.2 ± 20.8	$p = 0.014$

ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; WBC – white blood cells; Hgb – hemoglobin; GFR – glomerular filtration rate; SD – standard deviation; * – Kruskal Wallis test.

The most frequent symptoms at the time of diagnosis were the weight loss, back pain, fatigue, nausea, leg oedema, abdominal pain, new onset of hypertension and simultaneously abdominal and back pain (Table 1). The other symptoms were hydrocele, headache, appetite loss and weakness. The duration of symptoms before diagnosis ranged from 3–15 months.

Only 2 of 13 patients had an identified risk factor for RF (use of β blockers). Ten patients had a history of prior comorbidities: hypertension in 10, diabetes mellitus in 3 and hypothyroidism in two patients.

At presentation, 12 of 13 patients had ureteral obstruction which was bilateral in 11 of them. In 8 out of 12 patients with hydronephrosis, the ureteral obstruction was relieved by a placement of the DJS in 7 and PNS in one patient. These procedures were done in other hospital in 7 of these patients.

As a complication of this procedure, 6 patients (75%) had a urinary tract infection which was resolved by using the appropriate antibiotics.

Figure 1 shows the radiographic classification of the patient population at presentation. All patients had active disease. Twelve patients had renal dysfunction with a mean serum creatinine of 334 μmol/L (range was from 108–1022 μmol/L). Out of these, 9 patients presented as the acute renal failure which was oligoanuric in 5 (38.5%). One was treated with hemodialysis before the admission to our hospital. Initially, three patients already had chronic renal failure. They were admitted for recurrent disease, after the previous treatment with surgical and/or IS therapy.

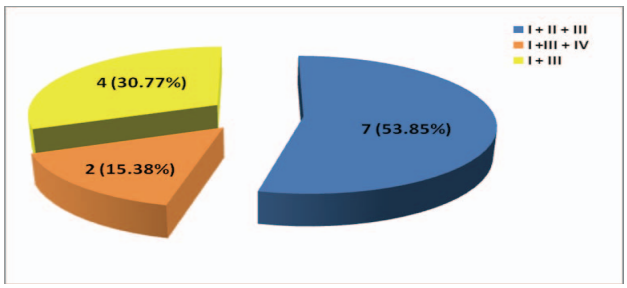

Fig. 1 – Radiographic classification at initial visit. Class I: soft-tissue density surrounding the infrarenal aorta and/or iliac vessels; Class II: soft-tissue density surrounding the infrarenal vena cava; Class III: lateral extension of the inflammation/fibrosis with compression of one or both ureters; Class IV: extension of fibrosis to include the renal hilum with compression of the renal artery and/or renal vein.

Table 2 shows the initial and follow-up laboratory data. ESR and CRP were elevated in 11 patients (range 26–140 mm/hr and 5.8–31 mg/L respectively). The mean hemoglobin level was 114 g/L (range 78–140 g/L).

Two patients with hypothyroidism had positive anti-TPO antibodies. Other autoantibodies were negative in all patients.

The remission occurred in all patients 12.7 ± 15.9 weeks (median 4 weeks) on average. The relief of pain and systemic symptoms was achieved in average 4 weeks.

The obstruction relief was observed after 4 weeks in 8 patients (61.5%), after 6 months in 10 (77%), and at the end of follow-up in 12 patients (92%). In 7 patients DJS and PNS were successfully removed, on average, 13 weeks after insertion (range from 3 to 32 weeks). In one patient, previously treated by ureterolysis, bilateral DJS were replaced by bilateral PNS due to the persistant obstruction, and was successfully removed after one year from one side. The other side required continued decompression due to the focal ureteral stricture for the total of 4 years. After that period, he stopped coming for the control examination and he was lost from the follow-up.

The ESR values declined from a mean 67.6 mm/hr to 26.3 mm/hr and the CRP values declined from the mean of 18.5 mg/L to 6.3 mg/L (Table 2, Figure 2).

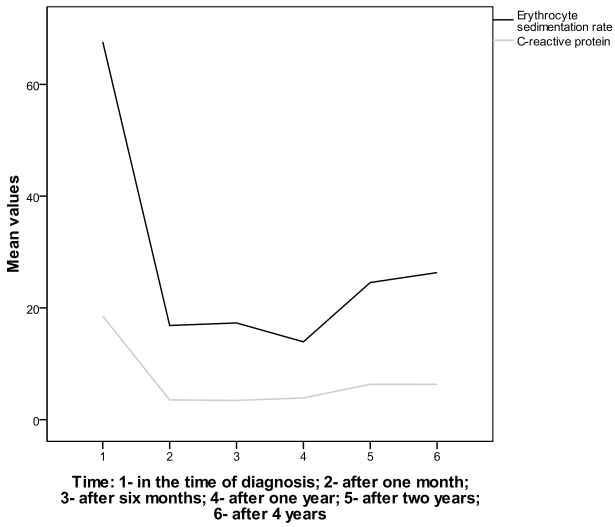

Fig. 2 – Erythrocyte sedimentation rate and C-reactive protein values during the 4-year follow-up.

The kidney function improved with increasing the GFR from the mean of 33.3 to 59.2 mL/min (Figure 3). In 4 patients, the chronic renal failure remained with GFR lower than 60 mL/min due to afunction of one kidney and in 3 patients with the chronic renal failure before treatment the renal function did not get worse.

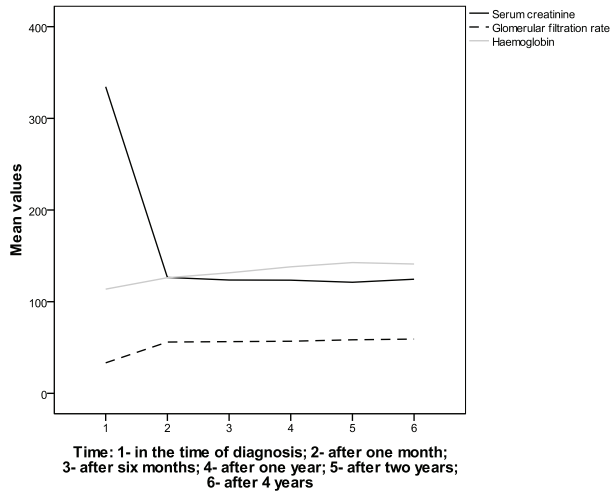

Fig. 3 – The serum creatinine, glomerular filtration rate and hemoglobin values during the 4-year follow-up.

All patients had a reduction of the fibrotic tissue on the MSCT/MR imaging. Seven patients had 100% of reduction in the periaortic mass, and the average percentage reduction was 76.9%. Figure 4 shows a representative baseline and follow-up MSCT scan of this study population.

The recurrence of disease was observed in 3 patients (23%). Two of them stopped the therapy after 6 and 31 months, respectively. The recurrence of the disease occurred 18 and 4 months after cessation, respectively. They were retreated, and one fully responded to the therapy while the second did not, and he received ureterolysis. In the third patient, the recurrence occurred after completing the protocol at the end of 48th month; she was retreated with complete remission.

There were no serious side effects of the treatment. Three patients with the previous diagnose of diabetes mellitus did not require change of the current therapy: 2 stayed on oral hypoglycaemic, and the third one was already on insulin therapy without a significant worsening of glycaemia.

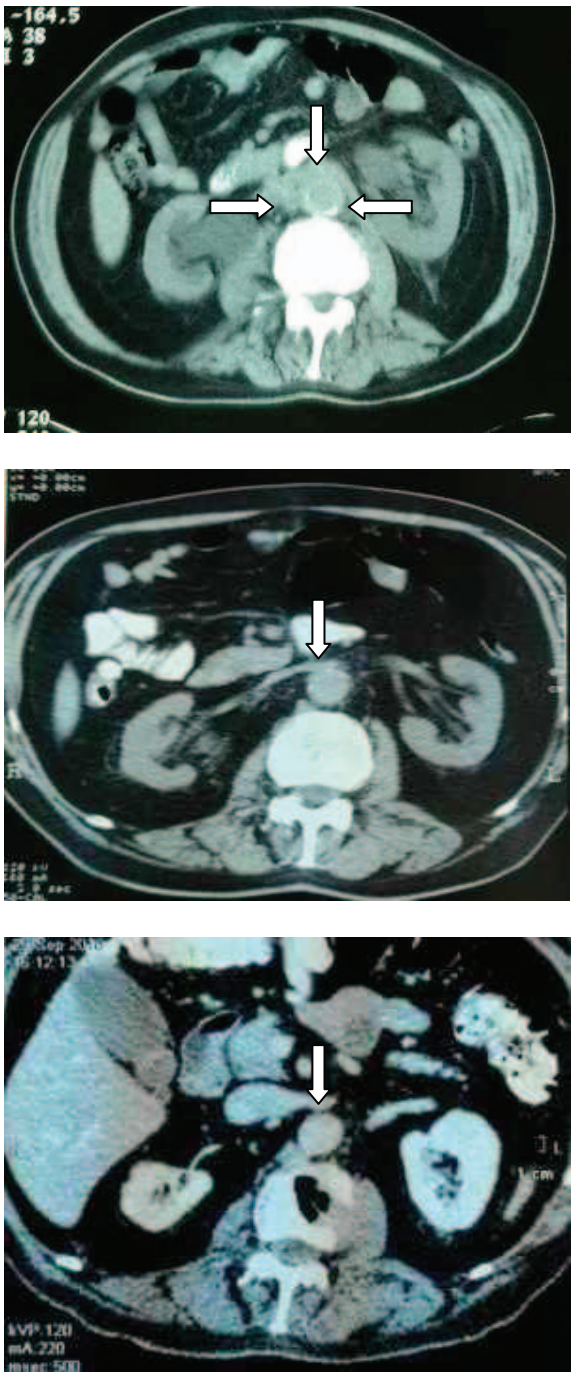

Fig. 4 – The reduction of the fibrotic tissue (Class I, II and III retroperitoneal fibrosis): A) before treatment; B) after 6 months and C) after 48 months of treatment.

Discussion

We retrospectively examined the medical outcome of 13 patients with IRF, receiving combined immunosuppressive therapy with corticosteroids and MMF.

The demographics of our patient population were similar to those of other reported series. Males were affected more often (61%) with diagnose made mostly in the fifth decade of life [14]. The most frequent constitutional symptom was the weight loss in 84.6% of the patients. The less frequent were the fatigue, nausea, appetite loss and weakness. The total of 92% of patients reported pain (back, abdominal, or both), which is consistent with other reports [9, 14, 15].

The etiology of IRF is not known, but several factors such as medications (β blockers, hydralazine, methysergide, ergotamine), surgery, radiation, infections and exposure to asbestos have been described as predisposing factors for developing retroperitoneal fibrosis, despite a scarcity of data to establish clear causal relationship [1]. In our study, two patients had a prior use of β blockers. This association was however described in the limited case reports in the relevant literature [16, 17]. Having in mind that the large number of patients take β blockers worldwide, we agree with some authors observation that this connection seems unlikely [14, 15, 18–20].

Additionally, one patient from our series decided to restart with β blockers after entering remission, and he did not experience recurrence of the disease for 74 months of follow-up.

Some studies reported frequent association of IRF with other autoimmune diseases (Hashimoto's thyroiditis, Graves' disease [21], ANCA-associated vasculitis [22], membranous nephropathy [23], rheumatoid arthritis [24], systemic lupus erythematosus [25], psoriasis [26]), or autoantibody positivity which emphasizes the autoimmune mechanisms in the pathogenesis of the disease [5]. ANA were the most frequent antibodies, detected in 60% of patients with IRF without evidence of connective tissue disease [5]. In our group, we observed two patients with Hashimoto's thyroiditis and positive anti-TPO antibodies. This is consistent with the previous observations that autoimmune thyroiditis is the most frequent autoimmune disease associated with IRF [1, 21]. Other autoantibodies (ANA, ANCA, RF) were negative in all patients.

Idiopathic retroperitoneal fibrosis is a progressive disease for which the consistent therapeutic recommendations have not been devised. Encasement of the ureters by retroperitoneal fibrous tissue leads to the obstructive nephropathy and serious complication including the end stage renal failure [1]. Because of the insidious clinical course and absent signs of impaired renal function until the late stage of the disease, about 75% of the patients had a renal failure and an irreversible shrinking of at least one kidney when diagnosis was made [27].

The treatment goals are to relieve ureteral obstruction, to stop the fibroinflammatory reaction and to prevent the recurrence of the disease.

Nowadays, a surgical treatment alone (ureterolysis with intraperitonealization and omental wrapping of the ureters) is not considered the first–line approach because of the high recurrence rate of the ureteral obstruction in up to 50% of patients [28–30]. Also, the surgical treatment has no effect on the systemic manifestation of the disease. As such, the conservative procedures – DJS, or PCN placement followed by a medical therapy is usually recommended, with surgery reserved for refractory cases [10, 31].

Considering the hypothesis that IRF represents a systemic autoimmune disease, various immunosuppressive (IS) agents were successfully used in the medical treatment of IRF. Some case studies and small series reported treatment with corticosteroids alone, or in a combination with cyclosporine, cyclophosphamide, methotrexate, azathioprine, MMF and antiestrogen drug tamoxifen [1, 9–13, 29, 32–36].

Corticosteroids are the most used IS drugs with the rapid improvement of the symptoms and obstruction relieve [28]. However, corticosteroids alone must be given in a high dose to control the inflammation, with the risk of known side effects. Additionally, there was no agreement in the literature about the dose and duration of the steroid therapy. Numerous duration of 6 weeks, 6 months and up to 2 years were proposed, but a significant number of patients have relapses after discontinuation of therapy and require additional treatment [10, 27, 29, 30, 37, 38]. To avoid relapses as well as to reduce the risk of side effects associated with a long-term intake of high doses, steroids were combined with other IS agents. The use of MMF was based on its known immunosuppressive and anti-fibrotic action [39, 40]. Combination of MMF and steroids was described in several case reports [41–44], small series [10] and a few larger series of 16 [40] and 31 patients [11]. In the study of prednisone and MMF, 89% of the patients had 25%, or a greater reduction in periaortic fibrotic mass with the average percentage reduction of 52.42%. The ureteral stents were successfully removed in 93% of obstructed ureters; only three patients (9.6%) had a recurrence of the disease. The duration of prednisone therapy in this study was 6 months, and MMF was given on average 23 months (range 6 to 63 months) [11].

In our study, the duration of steroid treatment was longer than in some published series [10, 27, 29, 37]. Our decision to use this approach was based on the observation that some patients needed a longer time (6 to 20 months) to achieve reduction of the size of the retroperitoneal mass [29], as well as on the reported high recurrence rate after discontinuation of steroids. The relapsing rate was observed in up to 72% of the patients [37], with the usual time of recurrence within 5 years after the diagnosis, although the rare case of recurrence was reported even after 9 and 10 years of follow-up [30, 45]. The recurrence of the disease appeared after a shorter time of steroid treatment (3–6 months) [10, 36] as well as after giving steroids for a year or longer [27, 29, 37]. The use of MMF, on the other hand, was limited by the fact that this drug is not registered for the treatment of IRF. Considering the significant number of relapsing patients, the usual time of the recurrence within 3–5 years, the serious complication of impaired renal function in this disease, and limitation in MMF use, we tried with steroid therapy for the total of 48 months, with faster initial tapering, in combination with MMF for 18 months.

The initial management of our patients depended on the level of renal impairment.

In cases of severe renal failure, with oligoanuria and elevated serum creatinine, DJS, or PNS are usually placed to achieve immediate upper urinary tract decompression. Although no guidelines exist, in a mild ureteral obstruction without the severe kidney function impairment, it seems advisable to start medical therapy without urinary drainage [27, 38, 46]. We treated 5 patients with the mild acute renal failure and preserved diuresis with i.v. corticosteroid pulses in 3 consecutive days with a rapid relief of the obstruction. This allowed avoidance of the complication with the DJS, or PNS placement, especially urinary tract infections which can be persistent and recurrent. In 8 of our patient with urinary drainage, 75% had urinary tract infection.

In all our patients, the combination of corticosteroids and MMF was successful in achieving the relief of the symptoms and correcting the laboratory abnormalities (ESR, CRP and haemoglobin level).

Whether the ESR and CRP levels are the reliable parameters for monitoring the disease activity is still unclear. Warnatz et al. [9] could not find a good correlation of disease activity with the CRP levels, but with the contrast enhancing lesions on CT, as well as Adler et al. [10] concerning that some patients had the normal CRP values despite radiologically detectable inflammation and a good response to the IS ther-

apy. Pelkmans et al. [47] found that the long-term decrease in ESR and CRP correlated with CT–documented mass regression. Like Scheel and Feeley [14], we observed a positive correlation of ESR as well as CRP with the disease activity.

All patients had reduction of the fibrotic tissue on MSCT/MRI, with the average 76.9% of reduction. Six patients did not achieve 100% of the mass reduction. The complete regression of the fibrotic tissue after therapy is very infrequent and a thin layer persists even in the patients who maintain complete remission. This residual mass, probably in most cases, represents metabolically inactive tissue [48].

Additionally, the kidney function improved and remained normal in 6 treated patients. In 4 patients the mild chronic renal failure remained due to afunction of one kidney. In 3 patients, with prior chronic renal failure, the renal function did not get worse. They all were previously treated for IRF with different strategies: by the first strategy – only with ureterolysis, disease was reoccurred after 4 months, by the second strategy – with ureterolysis and the IS agents (azathioprine and tamoxifen), the relapse occurred 33 months after cessation of IS therapy, and by the third one – with ureteral stenting for 12 months. None of the patients died during the follow-up.

The recurrence rate in our study was 23% (3 patients). Two patients did not finish the protocol and stopped the therapy after 6 months and 31 months, respectively. Third patient has completed the protocol and the recurrence occurred at the end of 48th month. This could indicate a need for longer duration of the treatment.

We did not observe the serious side effects of the treatment. In 3 patients with prior diagnose of diabetes mellitus the glycaemia did not worsen, and MMF was well tolerated without gastrointestinal, hematologic, or other abnormalities.

In this study, we used a radiographic classification system based on the anatomic location of the disease proposed by Scheel and Feely [14] which we found useful in making a correct diagnose and standardizing the extent of disease. Also, different classes could have different clinical outcomes, or complications. By definition, all patients should have class I disease. In our series, the majority of patients, 7 of them, had class I + II + III, 4 patients had class I + III, and 2 patients had I + III + IV class. The class IV usually had the lowest frequency, but these patients should be carefully monitored for the renal artery stenosis and had the endovascular stent placed, as it was required in one of our patients.

Study limitation

Our study, like many others, is limited by a small number of patients; the optimal management of IRF needs to be determined by prospective clinical trials in large patient cohorts.

Conclusion

Combined corticosteroid and the MMF therapy appears to be effective in restoring the renal function and reducing the fibrotic tissue in this small number of patients with IRF. It could prevent the need for the ureteral stenting and surgery. Longer treatment may reduce the possibility of recurrence. The long-term follow up is strongly recommended to estimate this regimen of treatment.

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Received on July 14, 2017.
Revised on November 17, 2017.
Accepted on January 09, 2018.
Online First January, 2018.

SHORT COMMUNICATION

UDC: 159.9:616-006
https://doi.org/10.2298/VSP171011004N

Empirical validation of the integrative psychological group intervention for women with breast cancer – preliminary results

Empirijska procena efekta psihološke grupne intervencije sa integrativnim pristupom kod žena sa karcinomom dojke – preliminarni rezultati

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Abstract

Background/Aim. Breast cancer diagnosis is an extremely stressful life event that brings a number of physical and psychological challenges. However, supportive and psycho-educational group interventions can significantly decrease psychological distress in patients. The aim of this study was to empirically validate the effects of the integrative psychological group intervention, regarding the affective state of women who underwent breast cancer surgery at the Oncology Institute of Vojvodina. **Methods.** This study was conducted on a sample of 30 women, with the average age of 53.17 years (standard deviation – SD = 10.09). Following the surgical intervention, the inpatients participated in an integrative group session consisting of the following parts: 1) supportive-expressive, 2) psycho-educational and 3) health-educational. Before the session, participants filled in a demographic data questionnaire, measures of positive and negative affect (PANAS), optimism (LOT-R), hope (AHS), neuroticism (BFI) and symptoms of depression (DASS-21). At the end of the group sessions, the participants filled in the PANAS again. **Results.** A paired-samples *t*-test showed that following an intervention, a statistically significant increase in positive affect had occurred ($t(29) = -4.44$, $p < 0.001$). For negative affect, the *t*-test also yields the statistically significant results ($t(29) = 5.60$, $p < 0.001$), showing that intervention led to a significant decrease in negative affect. The nonparametric Wilcoxon Signed-Rank test also confirmed these results. The multiple regression analysis ($F(4, 25) = 3.46$, $p = 0.02$) showed that high neuroticism and low symptoms of depression significantly predicted a greater increase in positive affect following the session. Another regression analysis ($F(4, 25) = 3.32$, $p = 0.03$) showed that the higher symptoms of depression and, marginally, higher hope significantly predicted a greater decrease in negative affect. **Conclusion.** Our results showed that the integrative psychological group intervention had positive short-term effects regarding the affective state of women who underwent breast cancer surgery, and that different psychological variables can play a significant role in prediction of changes in patients' affect.

Key words:
breast neoplasms; postoperative period; integrative oncology; psychotherapy group; surveys and questionnaires; women; treatment outcome.

Apstrakt

Uvod/Cilj. Dijagnoza karcinoma dojke predstavlja izuzetno stresan životni događaj koji donosi niz fizičkih i psiholoških izazova. Međutim, intervencije u vidu suportivnih ili psiho-edukativnih grupa mogu u značajnoj meri ublažiti psihološki distres kod bolesnika. Cilj istraživanja bio je da se empirijski validiraju efekti psihološke grupne intervencije sa integrativnim pristupom na afektivno stanje žena koje su operisane zbog karcinoma dojke na Institutu za onkologiju Vojvodine. **Metode.** Istraživanje je sprovedeno na uzorku od 30 ispitanica, prosečne starosti 53,17 godina [standardna devijacija (SD) = 10,09]. Nakon operacije, hospitalizovane pacijentkinje su učesvovale u integrativnoj grupnoj sesiji koja se sastojala od: 1) suportivno-ekspresivnog dela, 2) psiho-edukativnog i 3) zdravstveno-edukativnog segmenta. Pre učešća u intervenciji, pacijentkinje su popunile upitnik o demografskim podacima, meru pozitivnog i negativnog afekta (PANAS), skalu optimizma (LOT-R), nade (AHS), meru neuroticizma (BFI) i simptoma depresije (DASS-21), a na kraju grupne sesije, ponovo su popunile PANAS. **Rezultati.** *T*-test za zavisne uzorke pokazao je da, nakon učešća u psihološkoj grupnoj intervenciji, dolazi do statistički značajnog povećanja pozitivnog afekta ($t(29) = -4.44$, $p < 0.001$), kao i do statistički značajnog smanjenja negativnog afekta ($t(29) = 5.60$, $p < 0.001$).

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Neparametrijski Vilkoksonov test rangova takođe je pokazao da su dobijene razlike statistički značajne. Multipla regresiona analiza ($F(4, 25) = 3.46$, $p = 0.02$) pokazala je da povišen neuroticizam i sniženi simptomi depresije značajno predviđaju povećanje pozitivnog afekta. Druga regresiona analiza ($F(4, 25) = 3.32$, $p = 0.03$) pokazala je da žene koje imaju povišene simptome depresije i, marginalno značajno, višu nadu, ostvaruju izrazitije smanjenje negativnog afekta nakon intervencije. **Zaključak.** Rezultati studije su pokazali da integrativna psihološka grupna intervencija ima pozitivan kratkoročni efekat na afektivno stanje pacijentkinja operisanih zbog karcinoma dojke, te da različite psihološke varijable mogu igrati značajnu ulogu u predikciji promene na planu afektivnog stanja.

Ključne reči:
dojka, neoplazme; postoperativni period; onkologija, integrativna; psihoterapija, grupna; ankete i upitnici, žene; lečenje, ishod.

Introduction

Being diagnosed with breast cancer, one of the most common forms of malignant disease in women worldwide, represents an extremely stressful life event. Moreover, the long-lasting and demanding process of oncological treatment is an additional source of stress for the majority of patients. Facing the diagnosis and treatment of breast cancer brings not only numerous physical but also psychological challenges. Although the general prognosis for breast cancer nowadays is relatively good, the prevalence of psychological distress among breast cancer patients remains high, leading to an increased risk of developing serious symptoms of anxiety and depression as well as mood disorders [1–3]. Previous research has found that depression and anxiety are overall most prominent shortly after the diagnosis and that they mainly drop off during the treatment and over the time [4–6]. Various difficulties in adjustment to illness manifest not only through the anxiety and depression symptoms, but also via intense filings of anger, guilt, sense of worthlessness and hopelessness [7].

In order to reduce these adverse psychological effects of the illness, many oncology institutions organize the group therapy interventions for the patients. Although there is a plenty of group interventions which differ in their approach and basic philosophy, most of them can be specified as predominantly supportive or psychoeducational [8]. Supportive interventions are primarily focused on overcoming social isolation, sharing painful emotions, personal experiences and concerns, while psychoeducational groups include health education, stress management techniques, problem solving and various other coping strategies [8]. Some studies found that supportive groups were beneficial in diminishing the patients' emotional distress [9, 10]. Problem-solving focused training was also shown to significantly reduce emotional distress [11]. Moreover, when compared to supportive interventions, the psychoeducational groups were found to be more efficient in diminishing the symptoms of anxiety and depression, leading to improved coping style and better adaptation to illness [12]. One interesting study examined relative efficacy of group coping skills treatment, supportive group therapy and control group, for the patients with different cancer types [13]. In their research, the authors offered strong support for the efficacy of group treatment focused on skills training [13]. They found that the patients who took part in the coping skills training achieved positive changes regarding affect, work, physical and social activities, intimacy and sexuality, distress, communication and coping with medical procedures [13]. The same study revealed that the patients who received a supportive group therapy showed little improvement, while the functioning of the control patients deteriorated over time [13]. More recent studies which examined the long-term effects of supportive in contrast to the psychoeducational groups showed that both interventions resulted in improvement over the course of time [8]. However, psychoeducational groups showed the superior short-term effects in respect of the enhanced coping skills, but not long-term benefits [8]. While the majority of studies regarding group interventions in oncology setting were more interested in the question of which type of treatment works better, some research was more focused to the question of which kind of treatment is more beneficial for whom. Thus, one study explored the extent to which psychosocial variables moderated the effects of different types of group interventions for the breast cancer patients [14]. This study revealed that the educational groups showed greater benefits for the functioning of those women who entered the study with more difficulties, lacked social support and had scarce personal recourses, such as a low self-esteem, negative body image, or high illness uncertainty. The peer discussion (supportive) groups were more beneficial to those women who lacked medical and partner support, but showed to be harmful for the women who had already had high levels of social support [14].

All in all, the empirical data generally indicate that the group approach is, one way or another, beneficial for the women facing emotional distress due to breast cancer. Knowing that the group experience can be emotionally curative and socially strengthening, a standard procedure of psychological group support was likewise introduced and established at the Oncology Institute of Vojvodina. These interventions are designed as one-session meetings which are integrative and semi-structured in their nature, with elements both of supportive and educational approach.

The aim of our research was to empirically validate the short-term effects of these integrative group interventions on the emotional state of women who underwent breast cancer surgery. Firstly, we wanted to explore whether a participation in the group results in a short-term improvement regarding the emotional state of patients, and secondly, we wanted to examine if the psychological variables such as optimism, hope, neuroticism and symptoms of depression are significant predictors of possible changes in emotional well-being after the treatment. These variables were taken into account

knowing that the neuroticism and symptoms of depression are often regarded as vulnerability factors related to subjective experience of distress. On the other hand, there is a growing interest in constructs that aim to explain the process of positive thinking, with optimism and hope being considered as the factors of psychological resilience among the oncology patients.

Methods

Study design and procedure

This study was approved by the Ethics Committee of the Oncology Institute of Vojvodina. The research design was quasi-experimental with repeated measures, more precisely, a one-group pretest-posttest design. Although it is known that the quasi-experimental approach without the control group makes causal inferences more difficult, we decided to use this design due to the ethical issues. Since psychological groups have been previously established as a standard part of early psycho-oncology rehabilitation, it would be ethically problematic to exclude some participants who needed psychological support. Anyhow, knowing that the time interval between pretest and posttest measurements was short, the possibility of causal inferences was increased. In the second part of our study, the correlational approach was used.

The first measurement (pretest) was carried out on the second postoperative day, in the morning hours, during the inclusion of inpatients in the program of early psycho-oncology rehabilitation. Taking part in the research was voluntary, the participants were informed in detail about the purpose of the research and every individual signed an informed consent before entering the study. Before completing the questionnaires in the pretest, all the inpatients were checked for the severity of postoperative pain, or any other difficulties that could have a significant impact on their answers. If this was the case, the patients approached the research only after the symptoms were alleviated. Those participants who agreed to take part in a group intervention attended a one-session meeting. At the end of the session, the participants filled in the questionnaire again (posttest).

Sample

The study was conducted on the total of 30 female participants aged from 33 to 69 years [mean (M) = 53,17; standard deviation (SD) = 10,09] who were diagnosed with breast cancer and hospitalized for surgical treatment at the Clinic for Operative Oncology at the Oncology Institute of Vojvodina. The inclusion criteria were as follows: a) participants had never been diagnosed with a malignant disease before, and b) the presence of metastases was not registered at the time of psychological assessment.

In respect to the level of education, 46,7% of the participants had completed secondary school, 16,7% of them had graduated from a college, and 36,7% had a university degree. Furthermore, 30% of the patients reported that they were employed, 26,7% were unemployed, while 43,3% were retired. Regarding the marital status, 63,3% participants were married, 6,7% lived in a common-law relationship, 10% were divorced and 20% were widowed. Moreover, 93,3% of the participants had children. Regarding the place of residence, 23,3% participants lived in the countryside, 13,3% lived in a town, and 63,3% resided in a city. Of the total sample, 66,7% inpatients had already had some surgical interventions that were not related to malignant disease, while for other respondents this was the first operation.

Treatment

A group session lasted from 75 to 90 minutes, depending on the number of participants, which varied from 3 to 6 inpatients. The first part of the session was supportive-expressive and aimed at sharing personal experiences, emotions, disease-related attitudes and concerns. All participants had the opportunity to present their reflections or to give a feedback to one another. The next part of the session was psychoeducational and contained brief skills training, such as stress management, problem solving and assertive communication. The third part of the session was dedicated to the health education, promotion of healthy lifestyle and prevention of functional complications. The supportive and psychoeducational parts of the session were guided by a psychologist, while the health-educational segment was led by a specialist in physiatry and epidemiology.

Instruments

Basic demographic data questionnaire was designed by the authors in order to gather information about the age of participants, a place of residence, their level of education, working, marital and birth status. In addition, we collected the data about possible previous surgical interventions and the referential diagnosis with which the patients were admitted to the Clinic for Operative Oncology.

The Positive and Negative Affect Schedule (PANAS) [15] was used as a general measure of subjective distress. It is a self-reporting questionnaire which consisted of 20 items, 10 of which measure positive, and the other 10 negative affect. The participants responded to the items on a five-point Likert scale. Positive affect implied the presence of emotional experiences such as joy, excitement, enthusiasm, alertness, interest, etc. On the other hand, negative affect implied the presence of subjective distress and unpleasant emotional experiences such as fear, anxiety, guilt, hostility, etc. In this study, the participants were given a "state" version of the instrument, directing them to answer how they felt "right now, at a given moment". In this research, the PANAS was used as a pretest and posttest measure, while all the other questionnaires were administrated only in the pretest period of assessment.

The Life Orientation Test–Revised (LOT-R) [16] is an instrument used for evaluation of optimism as a dispositional personality trait. The individuals who achieved high scores on this scale had a greater tendency to believe that the good things were more likely to happen than the bad things [16]. This scale consisted of the total of 10 items (e.g. "In uncertain times, I usually expect the best"), 4 of which are fillers and

did not enter the total score. The items were answered on a five-point Likert scale.

The Adult Hope Scale (AHS) [17] is a self-report measure used for the assessment of hope as a relatively stable personality disposition. The instrument included two subscales corresponding to the Snyder's cognitive model of hope – Agency (representing a goal-oriented energy) and Pathways (standing for the perceived ways for reaching goals). The scale had the total of 12 items. Each of the two subscales had 4 items, while the remaining 4 items were fillers and did not enter the final score. In the original version of the questionnaire, the answers were given on an eight-point Likert scale. Nevertheless, in our study a four-point Likert scale was applied, in order to make answering more accessible to the senior participants.

The Big Five Inventory (BFI) [18] is a 44-item questionnaire, designed to measure the traits based on the Big-Five personality dimensions. For the assessment of neuroticism, we used the Neuroticism subscale from the BIF which consisted of 8 items with a five-point Likert scale. Higher neuroticism implied a tendency towards frequent experiences of anxiety, dysphoria, hostility, irritability, vulnerability, low self-esteem, etc. Diminished neuroticism represented the emotional stability.

The Depression Anxiety Stress Scales–21 (DASS-21) [19] is a self-report measure of three unpleasant emotional states: depression, anxiety and stress. In order to evaluate the symptoms of depression, we used the Depression subscale from DASS-21 which contained 7 items (e.g., "I felt that I had nothing to look forward to.") with answers presented on a four-point Likert type scale (with 0 meaning "never" and 3 meaning "almost always"). The total score on the Depression subscale varies from 0 to 21. This instrument is not a diagnosis-specific measure, but rather an indicator of emotional distress [20].

Results

The descriptive statistics and reliability coefficients for all the variables/scales in the study are presented in Table 1. It can be seen that the values of skewness and kurtosis for all variables are within the acceptable range, which is +/- 1.5 [21], except for negative affect in the posttest phase. The reliability of the scales, expressed by the Cronbach's alpha coefficient for all measures, is good to acceptable.

In order to answer our first research question – whether a participation in our group intervention leads to the statistically significant short-term improvement of patients' emotional state, we analyzed the differences between the measures of positive and negative affect in the pretest and posttest phases. We used both parametric and nonparametric statistical analysis with intention to overcome possible violation of normality assumption and to provide more powerful support for our findings. Using the IBM SPSS Statistics 21.0, two within-subjects (paired-sample) Sudent's *t*-tests were obtained. The independent variable was the measurement at two time points for one sample, while the dependent variables were the scores on positive/negative affect on the PANAS. For positive affect, a statistically significant *t*-test was obtained, meaning that there was a significant difference in the scores before (M = 31.50, SD = 7.75) and after (M = 35.83, SD = 7.36) a group intervention; $t(29) = -4.44$, $p < .001$. This result indicated that, as seen in Figure 1, the positive affect of inpatients increased at a statistically significant level after the group intervention. For negative affect, the *t*-test also yields the statistically significant results, showing that the difference in the scores before (M = 16.20, SD = 5.85) and after (M = 12.70, SD = 3.69) a group treatment was not likely to occur purely by chance; $t(29) = 5.60$, $p < 0.001$. More precisely, there was a statistically significant decrease in negative affect of inpatients after the participation in the group intervention. Reduction of negative affect after the group session can also be seen in Figure 1.

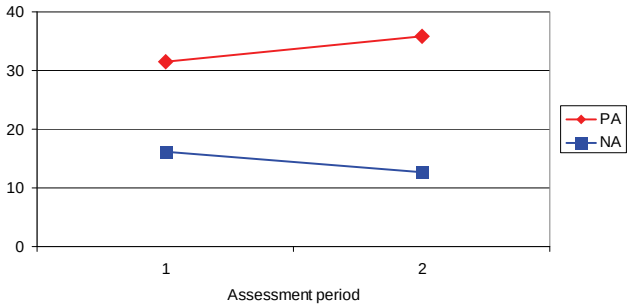

Fig. 1 – Mean scores for positive and negative affect in the pretest and posttest phases
PA – positive affect; NA – negative affect; 1 – pretest; 2 – posttest.

Table 1

Descriptive statistics and reliability coefficients for all the variables/scales

Variables	M	SD	Skewness	Kurtosis	Cronbach's alpha
Positive affect (pretest)	31.50	7.75	0.20	0.14	0.89
Negative affect (pretest)	16.20	5.85	0.84	-0.58	0.90
Positive affect (posttest)	35.83	7.36	-0.21	-0.78	0.89
Negative affect (posttest)	12.70	3.69	1.40	1.61	0.89
Optimism	24.60	3.88	-0.70	0.25	0.77
Hope	25.73	3,61	-0.81	0.58	0.84
Neuroticism	21.13	4.61	-0.23	-0.83	0.74
Depression	3.30	2.85	0.88	0.30	0.75

M – mean; SD – standard deviation

Table 2

Prediction of increase in positive affect by some psychological variables (Model 1)

Predictor variables	Standardized coefficient β	*t*	*p*
(Constant)		-0.64	0.53
Optimism	-0.16	-0.63	0.54
Hope	0.18	0.96	0.34
Neuroticism	0.56	2.32	0.03
Depression	-0.37	-2.08	0.05

Table 3

Prediction of decrease in negative affect by some psychological variables (Model 1)

Predictor variables	Standardized coefficient β	*t*	*p*
(Constant)		1.59	0.12
Optimism	-0.02	-0.06	0.95
Hope	-0.39	-2.01	0.05
Neuroticism	-0.40	-1.64	0.11
Depression	-0.38	-2.12	0.04

The smaller were the values of posttest-pretest difference, the greater was the decrease in negative affect

Furthermore, the nonparametric test, which did not make the normality assumption, was used in order to verify our results due to possible normality violation. The Wilcoxon Signed-Rank test is the most frequently used alternative to the paired sample *t*-test. The Wilcoxon Signed-Rank test indicated that the posttest scores on positive affect were significantly higher than the pretest scores, $Z = -3.63$, $p < 0.001$. The same test showed that the scores for negative affect in the posttest phase were significantly lower than the pretest scores, $Z = -4.22$, $p < 0.001$.

From the above-mentioned results, it can be seen that both parametric and nonparametric statistical approaches showed that the differences in positive and negative affect before and after a group intervention were statistically significant, and therefore likely to be caused by the treatment.

In order to answer our second research question, that is – which psychological variables are statistically significant predictors of change in positive and negative affect after the treatment, two multiple regression analysis were performed. In our research, the change in affect was defined as a difference between the posttest and pretest scores[1]. Hence, in the first regression model, the criterion variable was the difference in the posttest and pretest scores on positive affect, while the predictor variables were optimism, hope, neuroticism and symptoms of depression. In the second regression analysis, the criterion variable was the difference in the posttest and pretest scores on negative affect, while a set of predictors remained the same as in the previous model.

In the first regression analysis, a statistically significant model was obtained ($F(4, 25) = 3.46$, $p = 0.02$). The coefficient of determination (R^2) indicated that 36% of the variance of the criterion variable can be explained by the given model. In Table 2 we can see that neuroticism ($p = 0.03$) and, in negative direction, depression ($p = 0.048$) turned out to be the significant predictors of elevated positive affect after a group intervention. More precisely, our results showed that those individuals who were higher in neuroticism tended to achieve a greater increase in positive affect after the group session. However, those women who had more symptoms of depression tended to respond with less enhancement of positive affect.

In the second regression analysis, once again, a statistically significant model was obtained ($F(4, 25) = 3.32$, $p = 0.03$). The value of the coefficient of determination (R^2) indicated that 35% of the criterion variable's variance can be explained by the given model. In Table 3, we can see that a statistically significant predictor of change in negative affect following the group session was the level of depression symptoms ($p = 0.04$), and marginally significant, hope ($p = 0.05$). More specifically, those participants who had more prominent symptoms of depression and/or were higher in hope, showed greater reduction of negative affect after the group session. However, those women who did not have heightened symptoms of depression, nor highly expressed hope, had weaker effect of alleviation of unpleasant emotions after the group intervention.

Discussion

The findings of our research show that taking part in an integrative, one-session group intervention for women who underwent breast cancer surgery, may lead to an increased positive and decreased negative affect, to a statistically significant level. These results verified positive effects of an integratively formulated psychological group intervention, showing that the group experience with both supportive and educational content may indeed have a beneficial effect on the patients' emotional state on a short-term level. Our results are in line with findings of previous research which indicated that participation in a group intervention with the supportive and psychoeducational approach may reduce the

[1] For positive affect, the following rule was applied: the higher were the values of the posttest-pretest difference, the greater was elevation of pleasant emotions after the group intervention. For negative affect, the following rule was applied: the higher were the values of the posttest-pretest difference, the weaker was the decrease of unpleasant feelings after the group intervention; the smaller were the values, the greater was the decrease of negative affect.

emotional distress of cancer patients [9–11, 14], additionally emphasizing the importance of health education. The findings of this study provide a strong support for the notion that a psychological group support should be a standard part of inpatients' care after breast cancer surgery, especially knowing that the emotional difficulties are the most severe shortly after the diagnosis and in earlier phases of treatment [4–6].

Additionally, our research revealed some interesting findings regarding the prediction of change in the emotional state, following group treatment. It turned out that those patients with more severe symptoms of depression actually benefited more from the reduction in negative affect than from elevation in positive affect. This finding could be explained with the fact that trait anhedonia, which is one of the core symptoms of the depressive state, is associated with deficits in so called "hedonic response",i.e., the capacity for experience of pleasant emotions and satisfaction is reduced [22]. On the other hand, the participants with the low levels of symptoms of depression showed higher tendency towards an increase in positive affect and a poor decrease in negative affect, probably because they had already had low negative affect at the pretest measuring point.

Moreover, our research revealed a somehow unexpected finding – that those participants who were higher in neuroticism were more likely to have a greater increase in positive affect following a group session. However, some earlier studies on the general population have also shown that individuals who are high in neuroticism could improve their subjective well-being by increasing the positive emotions via certain cognitive strategies, rather than decreasing the negative emotions [23]. This finding is very promising, for it shows that negative effects of neuroticism, which is known to be an important risk factor for certain affective disorders [24], may be buffered at least in a short-term perspective.

Eventually, our findings suggest that women who are higher in hope tend to have a greater decrease in negative affect after the group intervention. It could be assumed that a participation in a psychological group intervention fosters a goal-oriented motivation and strategies [17], which in turn diminishes unpleasant mood.

Interestingly, optimism did not turn out to be a significant predictor of increase in positive affect, nor of decrease in negative affect. This finding could be explained by the assumption that the individuals high in optimism already had elevated levels of positive affect and low levels of negative affect, and therefore could not benefit that much from the group intervention.

To sum up, it seems that the benefits reflected in the improvement of emotional state via fostering positive emotions are most powerful for those individuals high in neuroticism and low in depression. On the other hand, for the women high in depression, the reduction in negative affect seems to be more beneficial. Possibly, group process activates the intrapersonal resources due to which the individuals high in hope manifest greater reduction in negative affect after the session.

As the results of our study are preliminary, future research should be conducted on a larger sample. Moreover, a wider range of psychological variables related to adaptation to disease (such as body image, self-efficacy and coping strategies) should be taken into account. Also, it would be recommendable to examine not only the short-term but also the long-term effects of such group interventions, with more sophisticated methodological approach.

Conclusion

The results of this study not only showed that the integrative psychological group intervention combining supportive and educational elements has the short-term benefits on the participants' emotional state but also shed some additional light on the predictive power of some important psychological variables with regard to the change in affect, following a group intervention. Our findings offer strong support to a well-known standpoint, that the group experience has „a healing effect" on emotional well-being of women who face breast cancer treatment, and therefore such interventions should be a standard part of patients' care in an oncology setting.

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Received on October 11, 2017.
Revised on December 18, 2017.
Accepted on January 08, 2018.
Online First January, 2018.

ORIGINAL ARTICLE

UDC: 616.248
https://doi.org/10.2298/VSP171114006M

Validation of the Serbian version of the Asthma Control Test

Validacija srpske verzije Testa za kontrolu astme

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Abstract

Background/Aim. Asthma still remains poorly controlled in the majority of patients. The Asthma Control Test (ACT) is a short and useful patient-administered questionnaire for identification of patients with poor asthma control in clinical settings. The aim of this study was to validate a Serbian version of the ACT in the adults with asthma. **Methods.** A total of 250 consecutive adult asthmatic patients were recruited in a prospective observational study. The exclusion criteria were chronic respiratory disease and acute respiratory tract infection in preceding 4 weeks. **Results.** The spirometry and ACT questionnaire were performed on the baseline visit and 6 months later. The ACT test was completed by 98.8% of patients with the mean time of completion of 4.5 minutes. The correlation of ACT score and lung function parameters (forced expiratory volume in 1 second – FEV_1 and forced vital capacity – FVC) was significant ($p = 0.016$ and $p = 0.002$, respectively). A change in the ACT scores between baseline and 6-months visit was not associated with a change in FVC and FEV1. The ACT score had excellent diagnostic accuracy according to the physicians asthma control classification and even outstanding accuracy according to the patients' classification. **Conclusion.** The results of this study confirm the reliability, validity and accuracy of Serbian version of the ACT, contributing to established value of original ACT test and with consistent findings as the previously reported validity of ACT in other languages. Therefore, it should be utilized more in everyday clinical practice as a useful and reliable tool of asthma control assessment.

Key words:
asthma; surveys and questionnaires; serbia.

Apstrakt

Uvod/Cilj. Astma i dalje ostaje slabo kontrolisana kod većine bolesnika. Test za kontrolu astme (*Asthma Control Test* – ACT) je kratak i koristan upitnik za identifikaciju bolesnika sa lošom kontrolom astme. Cilj ove studije je bio da se učini validacija srpske verzije ACT kod odraslih bolesnika sa astmom. **Metode.** Ovom prospektivnom opservacionom studijom je obuhvaćeno 250 odraslih bolesnika sa astmom. Kriterijumi za isključenje su bili postojanje hroničnog respiratornog oboljenja kao i akutna infekcija respiratornog trakta u prethodne četiri nedelje. **Rezultati.** Prilikom prve posete i šest meseci kasnije učinjena je spirometrija i popunjen ACT. ACT je popunilo 98,8% bolesnika sa prosečnim vremenom završetka od 4,5 minuta. Korelacija rezultata ACT i parametara plućne funkcije (forsirani ekspiratorni volumen u prvoj sekundi – FEV1 i forsirani vitalni kapacitet – FVC) bila je značajna ($p = 0,016$ odnosno $p = 0,002$). Promena rezultata ACT između prve posete i nakon šest meseci nije bila povezana sa promenom FVC i FEV1. Rezultat ACT je imao odličnu dijagnostičku tačnost u klasifikaciji kontrole astme prema lekaru i čak izuzetnu tačnost prema klasifikaciji samog bolesnika. **Zaključak.** Rezultati studije potvrdili su pouzdanost, validnost i tačnost srpske verzije ACT, doprinoseći već utvrđenoj vrednosti originalne verzije ACT. Takođe, ovi rezultati su u skladu sa rezultatima istraživanja validnosti verzija ACT na drugim jezicima. Stoga bi ga, kao korisno i pouzdano sredstvo za procenu kontrole astme, trebalo više koristiti u svakodnevnoj kliničkoj praksi.

Ključne reči:
astma; ankete i upitnici; srbija.

Introduction

Asthma is a heterogeneous disease characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms (wheeze, shortness of breath, chest tightness and cough) that vary over time and in intensity together with a variable expiratory airflow limitation [1]. Asthma is one of the most common chronic diseases worldwide with

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the estimated 300 million affected individuals. Prevalence of asthma in Belgrade, Serbia is 6.8% in adults [2]. The developed countries have high health care expenditure on asthma, especially when inadequately controlled [3].

The guidelines on diagnosis and management of asthma were previously focused on the assessment of severity of symptoms, limitation and variability of airflow, but their disharmony suggested that the severity alone, cannot be used for the assessment of therapy and classification [4]. On this basis, the revised Global Initiative for Asthma (GINA) guidelines, in year 2006, proposed a new classification based on the level of control rather than the severity of asthma [5]. Achieving and maintaining an adequate asthma control was defined as the primary goal of asthma management. When asthma is well-controlled, the patients have no night and day symptoms, lead active life, have little, or no need for reliever medication, have normal, or near-normal lung function and no exacerbations. These are the parameters that the doctors usually integrate to assess the asthma control and create adequate treatment plans.

Nevertheless, asthma still remained poorly controlled in a majority of patients [6, 7]. These circumstances alarmed the need for the development of tools that are easy and fast to administer in everyday practice, that identify the parameters that accurately assess control, and are simple to manage both by the patients and physicians. The Asthma Control Test (ACT) is a short and useful patient-administered questionnaire for identification of patients with poor asthma control in clinical settings, according to the GINA criteria [8, 9]. The ACT score is a valid tool to simply assess the current level of asthma control in terms of symptoms, rescue medication use, and (peak expiratory flow – PEF) variability, and it also correlated better than spirometry with the treatment decisions made by the asthma specialists (PEF and exhaled nitric oxide – NO) [10, 11].

Concerning the valuable role of ACT in the asthma control assessment, the aim of this study was to validate a Serbian version of the Asthma Control Test (ACT) in the adults with asthma.

Methods

This was an observational, prospective, multicentric study conducted from January 1st, up to December 31st, 2016, in two health institutions in Belgrade, the Clinic for Pulmonary Diseases, Clinical Center of Serbia and the Institute for Students Health Care. The patients over 18 years of age diagnosed with asthma according to the Global Initiative for Asthma (GINA) classification [1], who attended a routine check-up visit were included in the study. The exclusion criteria were chronic respiratory disease and/or acute respiratory tract infection in preceding 4 weeks. The Global Initiative for Asthma (GINA) classification was performed in order to separate the patients' disease status at the: well-controlled, partially controlled and uncontrolled asthma [1, 5].

The ACT consisted of 5 items with 5 response options evaluating different dimensions associated with asthma control in previous 4 weeks; the daily activity limitations due to asthma, the presence of day or night symptoms, the use of rescue medications, and the subjective perception of asthma control (Figure 1). The questions were scored from 1 (worst) to 5 (best). The sum of the scores classified asthma control as: uncontrolled asthma (< 19 points), controlled asthma (20–24 points), and optimal disease control (25 points) [9, 10].

The ACT questionnaire was used on two visits, at the baseline visit and 6 months later. During the first visit, asthma control was assessed according to the GINA guidelines [1]: the socio-demographic data (age, gender, educational status) were collected, the asthma diagnosis date recorded and classification of severity of disease according to the GINA criteria was made.

At both visits, the spirometry measurements were performed in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines [12] as well as lung auscultation (breath sound, expiration duration, wheezing). The data about current therapy modality was also collected. The patients' perception and the physicians opinion of asthma control were classified into 5 categories: total control, good, partial, poor, without any control. The patients categorized their asthma control status according to the question 5 in the ACT questionnaire, while the physicians categorization rely on the clinical and spirometry findings. The study protocol was approved by the Ethics Committees of Clinic for Pulmonary Diseases and the Institute for Students Health Care. The patients signed the informed consent, and the whole study was planned according to the ethical guidelines detailed in the Declaration of Helsinki (revised in 1983).

Statistical analysis

A sample size was calculated according to Rosner [13]. To power the study to 80%, 0.05 significance level and standard deviation in the ACT values of 4.0 from the previous pilot study produced the sample size of minimum 220 subjects. Assuming 10% drop-out rate, we finally included 250 subjects in this current validation study. The validity of the ACT was analyzed for its feasibility, reliability, transversal and longitudinal validity and predictive capacity.

Feasibility of the ACT was evaluated by the percentage of missing responses and the time required to complete the questionnaire. A percentage of completed questionnaires of more than 80% and a mean time of completion of less than 5 minutes were expected.

Transversal validity of the ACT questionnaire was assessed through the ACT relationship with the lung function parameters (forced expiratory volume in the first second – FEV1 (L, %), forced vital capacity – FVC (L, %), and auscultation assessment (quality of breath sound, wheezing and expiration quality). The correlation analysis was performed to test relation between the ACT and FEV1 (%), and the FVC (%) expectable correlations were low to moderate. The relation between the ACT scores and asthma symptoms was estimated through the auscultation method (quality of breath sound, wheezes and expiration duration). One way analysis of variance (ANOVA) was used to compare the differences in the sub-groups.

Test o kontroli astme

Ovaj test može pomoći osobama sa astmom da procene stepen kontrole astme.

Molimo Vas da zaokružite odgovarajući broj pored odgovora za svako pitanje. Ukupno ima PET pitanja.

Rezultat ovog testa ćete dobiti sabiranjem brojeva koji odgovaraju svakom Vašem odgovoru. Molimo Vas da prodiskujete rezultate sa Vašim lekarom.

1. U protekle 4 nedelje, koliko često Vas je astma ometala u obavljanju uobičajenih aktivnosti na poslu, u školi ili kući?

Uvek 1	Vrlo često 2	Povremeno 3	Retko 4	Nikada 5

2. U protekle 4 nedelje, koliko često ste imali osećaj nedostatka daha?

Češće od jednom dnevno 1	Jedanput dnevno 2	3-6 puta nedeljno 3	1-2 puta nedeljno 4	Nikada 5

3. U protekle 4 nedelje, koliko često su Vas simptomi astme (šištanje u grudima, gušenje, kašalj, pritisak u grudima) budili noću ili rano ujutru?

4 i više noći nedeljno 1	2-3 noći nedeljno 2	Jedanput nedeljno 3	Jednom ili dva puta 4	Nikada 5

4. U protekle 4 nedelje, koliko često Vam je bila potrebna pumpica za otklanjanje simptoma ili inhalator?

3 i više puta dnevno 1	1 ili 2 puta dnevno 2	2 ili 3 puta nedeljno 3	1 nedeljno ili ređe 4	Nikada 5

5. Kako biste ocenili kontrolu Vaše astme u protekle 4 nedelje?

Uopšte nije kontrolisana 1	Slabo kontrolisana 2	Donekle kontrolisana 3	Dobro kontrolisana 4	Potpuno kontrolisana 5

Fig. 1 – Asthma Control Test (ACT) – Serbian version.

Longitudinal validity was analyzed by testing the mean change in the ACT scores with changes in the clinical variables [FEV1 (%) and FVC (%)], which were categorized into 3 groups: reduction, without change and increase. The change in asthma control as perceived by the physician and patients was analyzed according to 5 categories questions.

Reliability analysis

The ACT questionnaire was estimated in terms of internal consistency and test-retest reliability. Internal consistency was measured by the Cronbach α statistic and test-retest reliability through the interclass correlation coefficient (ICC) [14]. The ICC greater, or equal to 0.70 was expected.

Predictive potential of the ACT score

The predictive potential of the ACT score compared to the clinical item (FEV1) for the asthma control prediction, perceived by the physicians and patients, respectively was performed by using the receiver operating characteristic (ROC) curve analysis. We aimed to test the diagnostic accuracy of FEV1, ACT score and model of integrated FEV1 and the ACT. The model formulated by the binary logistic regression analysis enabled the integration of FEV1 and ACT score and the areas under the receiver operating characteristic (AUC-ROC) curves with 95% confidence interval (CI) and *p* values were calculated.

According to the Hosmer and Lemeshow [15] (H-L) rules for the logistic models, the discriminative abilities of the

models were classified per their AUC values as poor ($0.5 \leq AUC < 0.7$), acceptable ($0.7 \leq AUC < 0.8$), excellent ($0.8 \leq AUC < 0.9$) and outstanding ($AUC \geq 0.9$). p value < 0.05 was considered a statistically significant. For all analyses, the SPSS software (IBM®SPSS®version 22.0) was used.

Results

A total of 250 consecutive adult asthmatic patients were recruited in a prospective observational study, but 3 patients did not complete the ACT at the baseline visit (3 participants gave up during the first visit). Table 1 presents the basic patients' data for the whole study group and the sub-groups, according to the GINA recommendations for the asthma status assessment. One hundred and eleven (45%) patients had intermittent, while 109 (44.1%) mild persistent, 19 (7.7%) moderate persistent and 8 (3.2%) severe persistent asthma.

The study sub-groups with different level of asthma control did not differ by gender distribution, age, level of education and asthma control. Our subjects had a minimum of secondary school education. It was obvious that the subject with uncontrolled asthma had the significantly lower pulmonary function parameters (FVC, FEV1 and FEV1/FVC), compared to the subjects with well-controlled disease and for several measures also to partially controlled subjects.

Feasibility

The ACT test was completed by 98.8% of patients with the mean time [standard deviation (SD)] of completion of 4.5 (4.0) minutes.

Reliability

The Cronbach α for the ACT was 0.81 for the whole study group. The test-retest reliability estimated by the ICC was 0.82 (95% CI 0.69–0.95).

Transversal validity

The correlation of ACT score and lung function parameters (FEV1 and FVC) was analyzed for transversal validity. According to the correlation coefficient values the correlation was significant, but low (Figure 2).

Table 1

The basic demographic and clinical data of asthma patients categorized according to the Global Initiative for Asthma (GINA) criteria [5]

Parameter	Whole group n = 247 (100.00%)	Well controlled asthma n = 54 (21.9%)	Partially controlled asthma n = 114 (46.1%)	Uncontrolled asthma n = 79 (32.0%)	*p*
Gender (male/female), n (%)	98/149 (39.7/60.3)	19/35 (35.2/64.8)	52/62 (45.6/54.4)	27/52 (34.2/65.8)	ns
Age (years), mean ± SD	25.4 ± 8.91	24.4 ± 8.01	25.9 ± 9.22	25.6 ± 9.10	ns
Education, n (%)					
secondary	230 (93.1)	51 (92.7)	104 (92.0)	75 (94.9)	ns
graduated	17 (6.9)	4 (7.3)	9 (8.0)	4 (5.1)	
Asthma duration (years), mean ± SD	12.2 ± 7.72	12.01 ± 7.30	12.09 ± 8.01	12.43 ± 7.66	ns
FVC (L), mean ± SD	4.74 ± 1.22	4.90 ± 1.14	4.88 ± 1.09	4.43 ± 1.11[a.b]	0.012
FVC (%), mean ± SD	106.6 ± 14.78	110.6 ± 16.73	107.2 ± 12.97	102.8 ± 14.75[aa]	0.008
FEV_1 (L), mean ± SD	3.71 ± 0.88	3.92 ± 0.82	3.82 ± 0.83	3.41 ± 0.93[aa.bb]	0.001
FEV_1 (%), mean ± SD	97.1 ± 17.23	102.9 ± 14.96	98.2 ± 14.19	91.4 ± 20.81[aa.b]	< 0.001
FEV_1/FVC, mean ± SD	78.3 ± 9.95	80.5 ± 6.99	78.6 ± 9.63	76.4 ± 11.75[a]	0.056

FVC – forced vital capacity; FEV_1 – forced expiratory volume in 1st second; SD – standard deviation. *p* from the ANOVA (post-hoc Tuckey test) or χ^2 test, where appropriate.

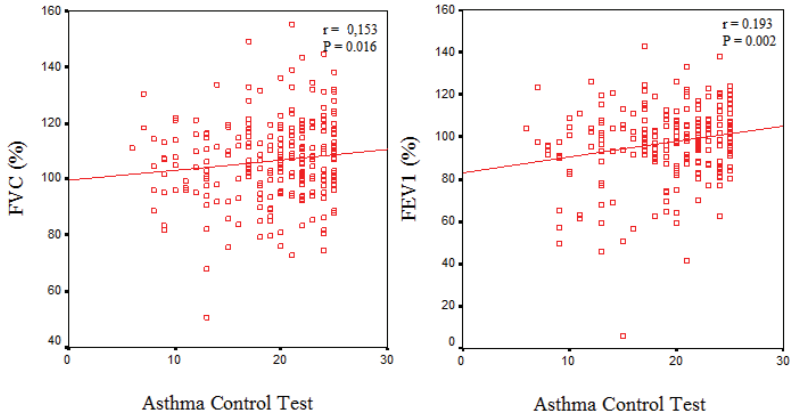

Fig. 2 – Correlation between the Asthma Control Test (ACT) and forced vital capacity (FVC) (%) and forced expiratory volume in 1st second (FEV1) (%) (the Pearson's parametric correlation).

Then we tested the ACT scores values in the sub-groups according to the auscultation measures: breath sound, wheezes and expiration duration. The results are presented in Table 2.

Table 2

The Asthma Control Test (ACT) scores according to lung auscultation (breath sound, wheezing and expiration quality)

Parameters of pulmonary function	mean ± SD	*p*
Breath sound intensity		
normal	19.3 ± 4.80	0.019
week	15.2 ± 5.67	
Wheezing		
without	20.2 ± 4.24	
low-tone	16.4 ± 5.39*	
high-tone	16.1 ± 6.50*	< 0.001
polyphone	14.4 ± 4.77***	
Expiration duration		
normal	19.7 ± 4.66	< 0.001
prolonged	17.0 ± 5.21	

*** $p < 0.05$; *** $p < 0.001$ compared respectively to normal pulmonary function regarding wheezing (without wheezing).**

The ACT scores were significantly lower (i.e., the worse disease control) in the patients with the worst lung auscultation findings.

Longitudinal validity

Table 3 presents the results of the mean change of the ACT scores in the subgroups by the level and direction – FVC and FEV1 were categorized as: reduction, without change and increase between two visits. The change in scores between baseline and 6-months visits was not associated with the change in FVC and FEV1 (% predicted).

Figure 3 shows the correlation of ACT score with physician's and patient's assessment of asthma control. According to both patients and physicians opinion, the ACT score was significantly lower in the patients with uncontrolled asthma. The asthma control assessment was equal to the physician's opinion in 145 (58.7%) patients.

Diagnostic accuracy (predictive potential) for the asthma control level

Table 4 presents the results of ROC analysis performed to test diagnostic accuracy of the FEV1 and ACT score, and its integrated Model, for the asthma control classification.

According to the H-L rules, the ACT score had an excellent diagnostic accuracy as stated by the physicians' asthma control classification and even outstanding accuracy according to the patients' classification. FEV1 had lower diagnostic accuracy (poor according to the H-L rules) by both classifications. The model of integrated both examined parameters (ACT and FEV1) did not improve the diagnostic accuracy neither to the physicians' nor to patients' classifications (Figure 4).

Table 3

The mean change in the Asthma Control Test (ACT) scores according to a change in clinical parameters of pulmonary function

Parameter	Reduction	Without change	Increase	*p*
FVC (%), mean ± SD	0.75 ± 11.62	-2.83 ± 3.37	3.06 ± 9.76	0.436
FEV_1 (%), mean ± SD	-1.87 ± 14.51	-10.0 ± 8.62	7.5 ± 23.54	0.163

FVC – forced vital capacity; FEV_1 – forced expiratory volume in 1st second.

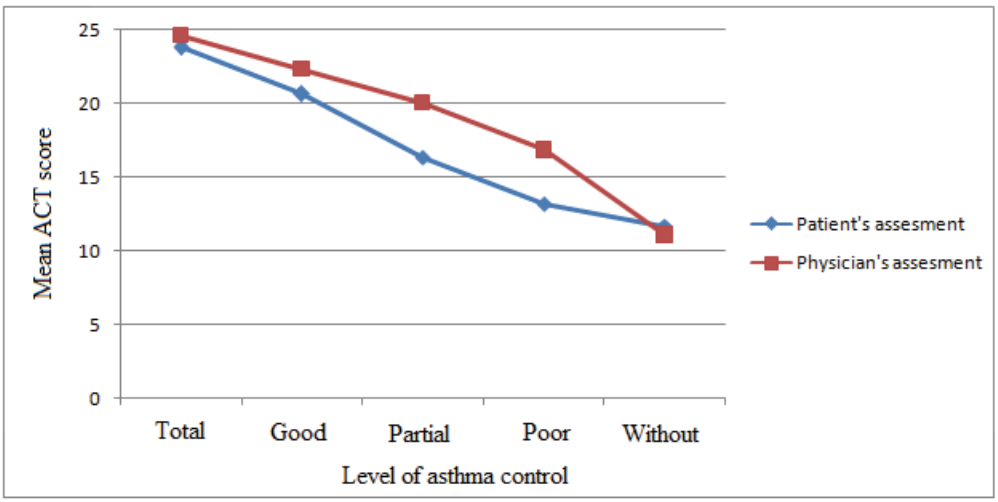

Fig. 3 – The mean Asthma Control Test (ACT) scores according to the level of asthma control as assessed by the patients and physicians.

Table 4

Diagnostic accuracy of the separate parameters and the Model of the integrated forced expiratory volume in 1st second (FEV_1) (%) and the Asthma Control Test (ACT) score according to the patients' and physicians' criteria for the asthma control classification.

Parameter	Physicians' classification		Patients' classification	
	AUC (SE)	95% CI	AUC (SE)	95% CI
ACT score	0.841 (0,025)	(0.792–0.890)***	0.936 (0.018)	(0.902–0.971)***
FEV_1 (%)	0.654 (0.036)	(0.585–0.725)***	0.627 (0.041)	(0.546–0.707)***
Model of integrated FEV_1(%) and ACT score	0.845 (0,025)	(0.796–0.895)***	0.891 (0.023)	(0.845–0.937)***

AUC – area under the curve, SE – standard error, CI – confidence interval.
***$p < 0.001$ according to the receiver operating curve (ROC) analysis.**

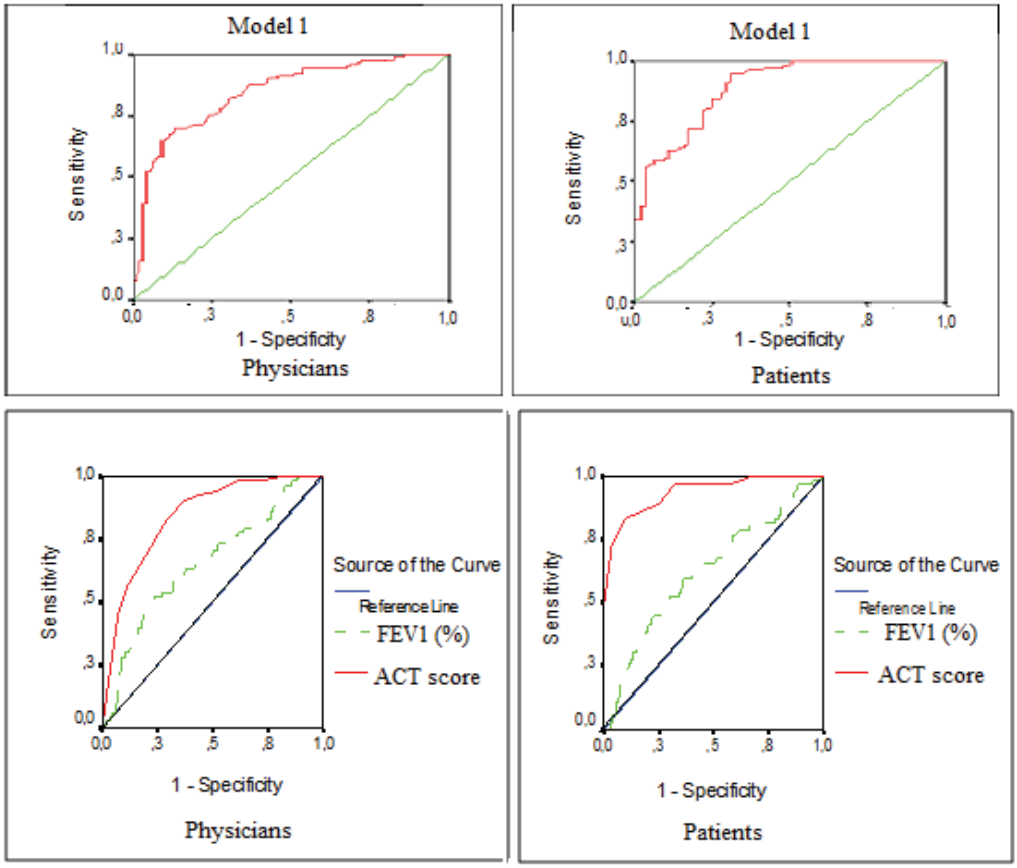

Fig. 4 – Receiver operating characteristic (ROC) analysis of the forced expiratory volume in 1st second (FEV_1), the Asthma Control Test (ACT) score and the Model 1 (integrated FEV1and the ACT score) in diagnostic capability for the asthma control prediction according to the patients' and physicians' criteria.

Discussion

Asthma, although heterogenic in nature, is a well-defined disease, however, the assessment and therefore the effective management of it is still elusive, so the equal emphasis is put nowadays both to asthma severity and asthma control [1, 5]. The Asthma Control Test has been developed and validated as a test of appropriate assessment of asthma control, responsive to changes in asthma control over time, proven to be useful tool to the clinicians and easy to apply in everyday clinical care [8, 16]. After validation of original ACT questionaire, the validation of ACT questionnaire its translations was reported in the last decade [17–22]. Although validation methodology was different in these studies, the overall findings regarding the translated ACT versions were consistent, confirming its value to assess asthma control. The spirometry testing is, on the other hand, the objective assessment of lung function status, but given the variable nature of asthma, it cannot be used as a sole predictor of disease status and control, and it is recommended to be combined with other tests for better asthma management [23].

In our research, in addition to the spirometric testing, we investigated the lung auscultation findings for transversal validity. Although more subjective parameter as lung function values, it is clinically relevant and performed in everyday practice for the estimation of asthma control. Our results

showed a significant correlation of asthma control assessment with the ACT and auscultation findings. The correlation of ACT score and lung function parameters was significant in our and the studies of Nathan et al. [8] and Gurková and Popelková [21], but not significant in the studies performed by Popovic-Grle et al. [22] and Lababidi et al. [18]. The only lung function parameter that was significantly associated with the ACT score was a peak expiratory flow (PEF) in Popovic-Grle et al. [22]. The correlation between the ACT and FEV1 was different in these studies probably because of the difference in the predicted values of patients FEV1 [8, 16, 17, 22].

For the testing of longitudinal validity, the highest increase in the ACT score was the most evident in the patients with the improved lung function measured by FEV1 and FVC. With the patients where decrease or no change in FEV1 and FVC was recorded, the ACT score was only slightly increased, or even decreased. Those changes were not statistically significant due to the large inter-individual variations of ACT scores. The finding is in accordance to the performed correlation analysis, which showed a significant positive relationship.

The ACT score was significantly positively correlated both in physicians' and patients' assessment of asthma control. We used the same 5-point scale of asthma control perception as in validation of original test (total, good, partial, poor and without control). Different scales were used by other authors [17], but all reports showed the positive and significant correlation of ACT score and the subjective assessment of asthma control. We did not test the difference among physicians' and patients' assessments in this research, the information which could contribute to the overall patient/physician relationship that was stressed out as important to the asthma management [1], because this important subject was not the aim of this study.

Our results showed an excellent and outstanding diagnostic accuracy of ACT score according to physicians' and patients' classification, and the result remain unchanged after integrating the lung function tests, as reported previously in the studies with the same methodology [17].

The reliability of Serbian translation was very similar to the original test as far as internal consistency was concerned (0.81 to 0.85 respectively), as well as to the version of ACT in other languages which were testing this parameter. The internal reliability of 5 questions in the ACT survey was 0.92 in the Arabic version, 0.87 in Czech's version, 0.85 in the original Nathan version, 0.83 in the Spanish version and 0.796 in Croatian version [8, 17, 18, 21, 22]. The Cronbach's α coefficient was slightly lower in the ACT performed by the North African population and it ranged from 0.58 for the Algerian ACT version to 0.67 for the Moroccan ACT version [19].

Conclusion

Almost 75% of asthma patients included into investigation were either partially controlled, or uncontrolled. This in itself suggest that the efforts for all aspects of asthma management should be continued.

The feasibility result of the Serbian version shows that the ACT test is easily applicable, understandable and not time-consuming. Overall results of our study also confirm that the reliability, validity and accuracy of Serbian translation contribute to the established value of original ACT questionaire and its versions in other languages. Therefore, it should be applied more in everyday clinical practice of respiratory physicians as a useful and reliable tool for the asthma control assessment.

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Received on November14, 2017.
Revised on January 04, 2018.
Accepted on January 11, 2018.
Online First January, 2018.

ORIGINAL ARTICLE

UDC: 616.348/.351-006-07
https://doi.org/10.2298/VSP171201007J

Fecal galectin-1 as a potential marker for colorectal cancer and disease severity

Fecesni galektin-1 – potencijalni marker kolorektalnog karcinoma i težine bolesti

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Abstract

Background/Aim. Colorectal cancer (CRC) represents one of the most common cancers worldwide. CRC is frequently diagnosed at advanced stages with poor prognosis, indicating the need for new diagnostic and prognostic markers. The aim of this study was to determine systemic and fecal values of galectin-1 (gal-1) and ratios between gal-1 and proinflammatory cytokines: tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interferon gamma (IFN-γ), in the patients with CRC and the relationship with clinicopathological aspects of the disease. **Methods.** The blood samples and feces liquid fraction of 58 patients with CRC were analyzed. The serum and fecal levels of TNF-α, IL-1β and IFN-γ and gal-1 were measured using sensitive enzyme-linked immunosorbent assay (ELISA) kits. **Results.** The fecal level of gal-1 was increased in the CRC patients with higher nuclear grade and poor tumor tissue differentiation. The gal-1/TNF-α ratio in the serum and feces had a higher trend in the patients with the advanced tumor-node-metastasis (TNM) stage as well as the detectable lymphatic and blood vessel invasion. The gal-1/TNF-α and gal-1/IFN-γ ratios were increased in the serum of patients with presence of lung/liver metastasis or peritoneal carcinomatosis, while the enhanced gal-1/IL-1 ratio was detected only in the serum of patients with lung metastasis. A positive correlation between the gal-1 value in feces and histological differentiation of tumor and biomarkers alpha-fetoprotein (AFP) and cancer antigen-19-9 (CA 19-9), respectively, was also observed. The fecal values of gal-1 higher than 13,708.29 pg/g presented a highly sensitive and specific marker for histological differentiation of tumor tissue. **Conclusion.** We believe that the predomination of gal-1 over pro-inflammatory cytokines TNF-α, IL-1β and IFN-γ in the patients with advanced and progressive CRC may implicate on an immunomodulatory role of gal-1 in the limiting ongoing proinflammatory processes. The fecal values of gal-1 can be used as a valuable marker for the severity of CRC.

Key words:
colorectal neoplasms; carcinoma; feces; galectin-1; disease progression.

Apstrakt

Uvod/Cilj. Kolorektalni karcinom (*colorecral carcinoma* – CRC) je jedan od najučestalijih karcinoma na svetu. CRC se često dijagnostikuje u uznapredovalim stadijumima sa lošom prognozom, ukazujući na potrebu za novim dijagnostičkim i prognostičkim markerima. Cilj ove studije bio je utvrđivanje sistemskih i fekalnih vrednosti galektina-1 (gal-1) i odnosa između gal-1 i proinflamacijskih citokina: faktoru nekroze tumora alfa (TNF-α), interleukina-1 beta (IL-1β) i interferona-gama (IFN-γ) kod bolesnika sa CRC i odnosa sa kliničko-patološkim aspektima bolesti. **Metode.** Analizirani su uzorci krvi i tečne frakcije fecesa 58 bolesnika sa CRC. Serumski i fekalni nivoi TNF-α, IL-1β, IFN-γ i gal-1 su mereni korišćenjem senzitivnog *enzyme-linked immunosorbent assay* (ELISA) testa. **Rezultati.** Fekalni nivo gal-1 povećan je kod bolesnika sa CRC sa velikim nuklearnim gradusom i slabo diferentovanim tumorskim tkivom. Odnos gal-1/TNF-α u serumu i fecesu značajno je veći kod bolesnika sa uznapredovalim tumor-nodus-metastaza (TNM) stadijumom, kao i detektabilnom invazijom limfnih i krvnih sudova. Odnosi gal-1/TNF-α i gal-1/IFN-γ su povećani u serumima bole-

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snika sa metastazama u plućima/jetri ili peritonealnom karcinomatozom, dok je povećan odnos gal-1/IL-1 detektovan samo u serumu bolesnika sa metastazama u plućima. Takođe, primećena je pozitivna korelacija između vrednosti gal-1 u fecesu i histološkog tipa tumora i biomarkera alfa-fetoproteina (AFP) i *cancer antigen* 19-9 (CA 19-9). Vrednosti gal-1 u fecesu veće od 13,708.29 pg/g predstavljaju visoko osetljiv i specifičan marker za histološku diferencijaciju tumorskog tkiva. **Zaključak.** Naši rezultati ukazuju na to da predominacija gal-1 nad proinflamacijskim citokinima, TNF-α, IL-1β, IFN-γ, kod bolesnika sa uznapredovalom i progresivnom bolešću ističe imunomodulatornu ulogu gal-1 u ograničavanju proinflamacijskih procesa. Vrednosti gal-1 u fecesu mogu se koristiti kao marker procene težine kolorektalnog karcinoma.

Ključne reči:
kolorektalne neoplazme; karcinom; stolica; galektin-1; bolest, progresija.

Introduction

Colorectal cancer (CRC) is one of the most common cancers and the fourth cause of cancer-related deaths [1]. Despite the constant achievements in the understanding of cancer biology, the morbidity and mortality rates of CRC continue to increase [1]. In most cases, the CRC is diagnosed at the advanced stages with poor prognosis. This phenomenon highlights the need for new diagnostic and prognostic markers. There has been a sustained interest in the identification of bio-markers for the prognosis and progression of CRC [2–4]. New markers should contribute to the prediction of prognosis, or relapse after therapy. Today, serum markers for CRC are preferred over tissue, or stool-based assays, especially for screening and monitoring purposes, which require repeat testing [4]. Novel studies point to the significance of fecal markers measurement in the detection and prediction of disease severity [5–7].

A large body of evidence indicates that galectins participate in a variety of normal cellular functions, and are disregulated in CRC [8–11]. Among all known galectins, galectin-1 (gal-1) is well characterized. gal-1 is a multifunctional β-galactoside-binding lectin produced by a variety of vascular, interstitial, epithelial, immune cells as well as neoplastic cells [12, 13]. It can be located either inside the cells in nucleus and cytosol, or in the extracellular space [12, 13]. It is shown that gal-1 is involved in several biological processes and in various phases of tumorogenesis such as regulation of cell growth and migration, cell-extracellular matrix and cell-cell interactions, angiogenesis, tumor–immune escape [14, 15]. An elevated expression of gal-1 was observed in tissues of various solid malignant tumors, whereas low, or no expression was found in the normal tissues [8–11]. The immunomodulatory role of gal-1 is also known, and its strong influence on inflammation is well-established [16].

The aim of this study was to evaluate the systemic and fecal values of gal-1 and ratios between gal-1 and proinflammatory cytokines in the patients with CRC and the relationship with clinicopathological aspects of disease. In this study, we demonstrate the enhanced fecal concentration of gal-1 in the CRC patients with higher nuclear grade and poor tumor tissue differentiation, while the predomination of gal-1 over proinflammatory cytokines in the patients with advanced tumor-node-metastasis (TNM) stage and metastatic disease.

Methods

Ethical approvals

The study was conducted at the Clinical Center in Kragujevac, Serbia, and the Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia, after the study protocol had been approved by relevant Ethics Committees of the Clinical Center in Kragujevac, Serbia, and Faculty of Medical Sciences, University of Kragujevac, Serbia. All patients gave their informed consent. All research procedures were made according to the Principle of Good Clinical Practice and the Declaration of Helsinki.

Subjects

Fifty-eight patients with CRC were enrolled in the study. All patients received surgical resection for CRC. A diagnosis was based on the endoscopic and histological criteria. The exclusion criteria included no well-defined pathology, no adequate clinical documents available and previous treatment with radiation and chemotherapy. The clinical data about age, gender, size of cancer, metastasis, and pathologic reports (vascular invasion, lymph node invasion, nuclear grade and well and moderate + poor differentiation) and a clinical stage by TNM were recorded and analyzed in the study. The blood and stool samples were taken before the surgery and stored at -80°C until enzyme-linked immunosorbent assay (ELISA**).**

Feces liquid fraction preparation

The stool samples (1–10 g) were collected in the morning in the sterile containers and weighed. One gram of fecal samples was diluted, mixed, homogenised in 5 mL of protease inhibitor cocktail (SIGMA, P83401), and then centrifuged, as previously described [17, 18]. The supernatant fluid was collected and stored at -80°C until ELISA.

Evaluation of tumor markers in serum

The serum levels of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), and cancer antigen 19-9 (CA19-9) were routinely determined by chemiluminescence enzyme

immunoassay (CLIA) in the central biochemical laboratory of the Clinical Center in Kragujevac.

Determination of galectin-1, TNF-α, IL-1β and IFN-γ in serum and feces

The serum and fecal concentrations of gal-1 and cytokines were measured, as described [19], by using the sensitive ELISA kits (R&D Systems, Minneapolis, MN, for gal-1, enzyme-linked immunosorbent assay tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interferon-gamma (IFN-γ); measurement of cytokines according to the manufacturer's instructions). Briefly, the 96-well plates were coated with capture antibody, overnight. The plates were washed with a washing buffer (0.05% Tween-20 in PBS), and incubated with blocking buffer (1% bovine serum albumin in PBS) for 1 hour at room temperature. The serum/fecal samples, or the standard recombinant gal-1/TNF-α/IL-1β/IFN-γ were introduced to the plates for 2 hours before the application of biotinylated detection antibody for 1 hour at room temperature. After introduction of streptavidin peroxidase for 1 hour, the plates were developed with substrate reagent for 20 minutes. The reaction was stopped by adding 4 mol/L sulfuric acid, and the absorbance was read at 495 nm by a microplate reader. We measured the exact concentration of mentioned biomarkers by intrapolation of a standard curve made by a series of well-known concentrations as per manufacturer's instruction. The values of measured cytokines were presented as pg/mL of serum and pg/g of feces, respectively.

Statistical analysis

The statistical analyses were performed by using the SPSS 20.0 software. The results were reported as the mean and standard error (SE). A statistically significant difference between the means of two groups was determined using the Student's *t*-test for the independent samples if the data had normal distribution, or Mann-Whitney *U*-test for the data without normal distribution. The Pearson's or Spearman's correlation, where appropriate, evaluated the possible relationship between the cytokines and disease severity and progression in the patients with CRC. The numerical values were assigned to different histological differentiation stages (well = 1; moderate + poor = 2). A strength of correlation was defined as negative or positive, weak (-0.3 to -0.1, or 0.1 to 0.3), moderate (-0.5 to -0.3 or 0.3 to 0.5), or strong (-1.0 to -0.5 or 1.0 to 0.5). *p*-value of 0.05 was considered as a statistically significant.

Results

Fifty-eight patients with CRC were enrolled in the study. There was no significant difference in the gender distribution (34 males and 24 females). The patients were similar in age (mean age 66 ± 1 years). The clinical and pathological characteristics of these patients are presented in Table 1.

Table 1

Baseline characteristics of patients

Characteristics	Values
Gender (male/female), n	34/24
Age (years), mean (range)	66 (50–82)
Site (P/D/R), n	14/34/10
Nuclear grade (I/II/III), n	7/37/14
Stage (TNM: I/II/III/IV), n	32/0/14/12
Necrosis (well/moderate/absent), n	15/43/0

P – proximal colon; D – distal colon; R – rectum; TNM – tumor-node-metastasis; n – number.

The serum and fecal concentration of gal-1 and a ratio between gal-1 and pro-inflammatory mediators, with regard to histopathologic characteristics of CRC

The patients with CRC were categorized into 3 groups according to the nuclear grade of tumor tissue: I, II and III, and analyzed for the systemic and fecal values of ratio between the gal-1 and pro-inflammatory mediators (TNF-α, IL-1β and IFN-γ). As shown in Figure 1A, the CRC patients with a higher nuclear grade appeared to have the higher fecal level of gal-1 (III *vs* II: 21,936.14 ± 3,601.19 *vs* 13,286.97 ± 782,97 pg/mL; $p = 0.020$; III *vs* I: 21,936.14 ± 3,601.19 *vs* 1,5724.30 ± 1,903.49 pg/mL; $p = 0.047$), systemic value of gal-1/TNF-α ratio (III *vs* II: 60.46 ± 9.01 *vs* 27.17 ± 2.62; $p = 0.009$; III *vs* I: 60.46 ± 9.01 *vs* 24.44 ± 0.89; $p = 0.032$), as well as the fecal gal-1/IFN-γ ratio (III *vs* II: 13.64 ± 0.78 *vs* 9.76 ± 1.39; $p = 0.001$; III *vs* I: 13.64 ± 0.78 *vs* 10.03 ± 2.96; $p = 0.048$).

Further, we classified the CRC patients into two groups, according to the histological differentiation rate: well and moderate + poor. In the patients with the poor tumor tissue differentiation, we detected the increased fecal gal-1 (moderate and poor *vs* well: 19,353.69 ± 2,224.35 *vs* 12,757.56 ± 1,207.58 pg/mL; $p = 0.026$) and the systemic gal-1/TNF-α ratio (moderate and poor vs well: 503,57 ± 100,01 *vs* 69.73 ± 11,61; $p = 0.042$; Figure 1B).

The serum and fecal gal-1/TNF-α ratios are associated with the TNM system and lymph and blood vessels invasion

The patients with CRC were divided into two categories on the basis of TNM stage of disease: I+II (localized tumor) and III+IV (metastatic tumor). There were no patients with TNM stage II. The patients with the TNM stages III+IV revealed a significantly higher gal-1/TNF-α ratio in the serum (115.03 ± 20.10 *vs* 60.51 ± 7.95; $p = 0.046$) and feces (16.84 ± 0.92 vs 10.36 ± 1.36; $p = 0.024$; Figure 2A).

The patients with CRC were divided into two groups, based on the presence of lymphatic/blood vessel invasion, respectively (+ and -). The increased gal-1/TNF-α ratio in the serum was detected in the patients with detectable lymphatic (146.95 ± 28.91 *vs* 58.53 ± 24.87; $p = 0.049$) and blood vessel invasion (38.62 ± 4.01 *vs* 22.82 ± 3.25; $p = 0.040$; Figure 2B).

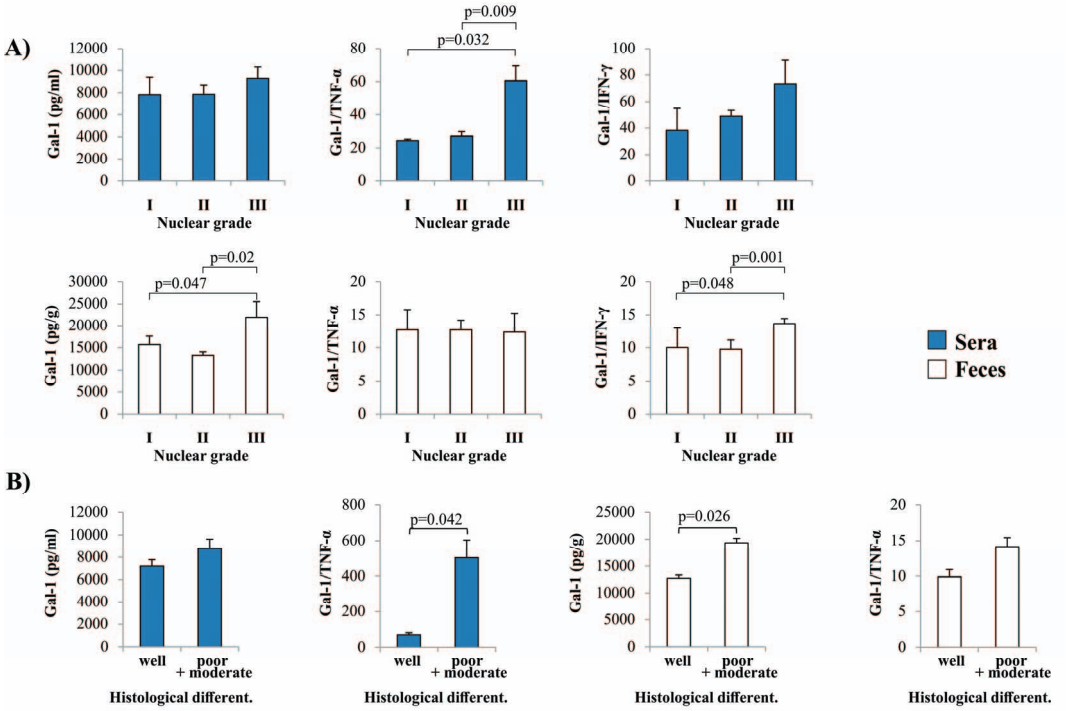

Fig. 1 – The serum and fecal values of galectin-1 (gal-1) and mediators of inflammation and their ratio in patients with colorectal cancer (CRC), based on histopathological characteristics of tumor.
A) The increased concentration of gal-1 and the gal-1/IFN-γ ratio in feces and gal-1/tumor necrosis factor (TNF)-α ratio in the serum of patients with higher nuclear grade of CRC. The patients with CRC were divided into three groups, based on a nuclear grade (I, II and III). The serum and fecal levels of all mentioned biomarkers were determined by enzyme linked immunosorbent assay (ELISA). The gal-1/interferon-gamma (IFN-γ) and the gal-1/tumor necrosis factor alpha (TNF-α) ratios were evaluated for each patient, separately.
B) The increased concentration of gal-1 in feces and the gal-1/TNF-α ratio in the serum of patients with poor histological differentiation of CRC. The patients with CRC were divided into two groups, according to a histological differentiation rate (well and moderate + poor). A statistical significance was tested by the Mann–Whitney Rank Sum test, or the independent samples *t*-test, where appropriate.

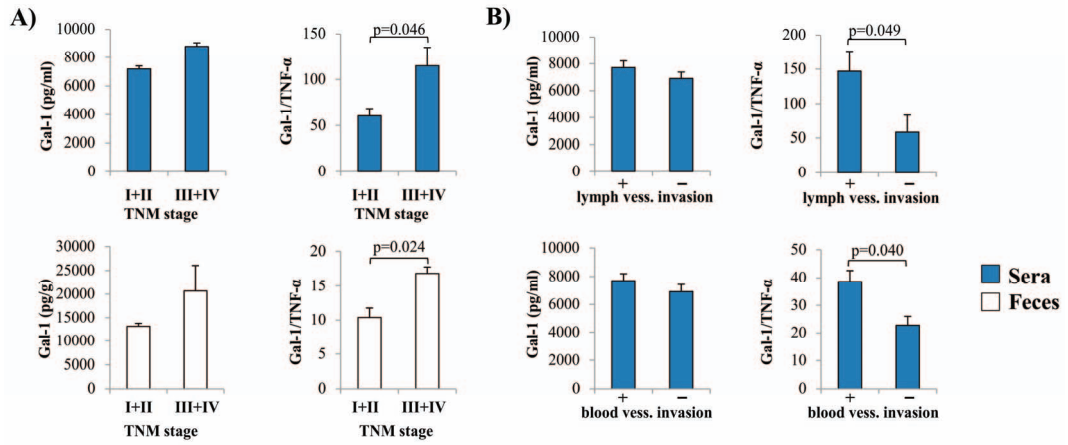

Fig. 2 – The concentrations of galectin-1 (gal-1) and gal-1/tumor necrosis factor (TNF) ratio in the serum and feces of patients with colorectal cancer (CRC), based on clinicapathological characteristics of tumor.
A) The increased concentration of gal-1/TNF-α ratio in the serum and feces in the patients with a higher tumor-node-metastasis (TNM) stage of CRC. The patients with CRC were divided into two groups, based on a TNM stage (I+II and III+IV). The serum and fecal levels of all mentioned biomarkers were determined by enzyme linked immunosorbent assay (ELISA). The gal-1/TNF-α ratio was evaluated for each patient, separately.
B) The increased gal-1/TNF-α ratio in the serum of patients with detectable lymphatic and blood vessel invasion of CRC. The patients with CRC were divided into two groups, based on the presence of lymphatic/blood vessel invasion (+ and -). The serum levels of all mentioned biomarkers were determined by ELISA. The gal-1/TNF-α ratio was evaluated for each patient, separately. A statistical significance was tested by the Mann–Whitney Rank Sum test, other independent samples *t*-test, where appropriate.

Liver, lung and peritoneal metastasis are associated with a higher gal-1/TNF-α ratio

Further, we divided patients into two categories based on presence of lung/liver metastasis, or peritoneal carcinomatosis, respectively. A higher gal-1/TNF-α ratio was found in the serum of patients with detectable liver metastasis (48.53 ± 6.95 *vs* 28.12 ± 2.87; $p = 0.005$), lung metastasis (70.61 ± 10.09 vs. 28.87 ± 2.51; $p = 0.001$), or peritoneal carcinomatosis (53.79 ± 11.42 vs. 29.71 ± 2.72; $p = 0.012$), in comparison to the patients without metastasis/carcinomatosis (Figure 3). In addition, we also found a higher gal-1/IFN-γ ratio in the serum of patients with detectable liver metastasis (72.68 ± 12.51 vs. 46.01 ± 3.26; $p = 0.043$), lung metastasis (100.34 ± 25.82 vs. 55.02 ± 5.25; $p = 0.033$), or peritoneal carcinomatosis (89.57 ± 19.57 vs. 54.65 ± 5.46; $p = 0.033$), as illustrated in Figure 3. An increased gal-1/IL-1 ratio was detected in the serum of patients with detectable lung metastasis (1,001.91 ± 82.09 vs. 791.65 ± 31.63; $p = 0.027$; Figure 3).

The Spearman's correlation analysis of gal-1 concentration in stool uncovered a positive correlation between the gal-1 value and histological differentiation stage of tumor (r = 0.357; $p = 0.025$). Further analyses also found that fecal gal-1 significantly correlated with the AFP levels (r = 0.317; $p = 0.028$), the CA 19-9 levels (r = 0.296; $p = 0.049$), but there was no significant correlation found with the CEA levels (Figure 4). The serum gal-1 did not correlate with same parameters and markers of colon cancer (data not shown). The analysis also showed that the fecal gal-1 can be a valuable marker for distinguishing poor and moderate differentiation of tumor tissue (Figure 4). The optimal cut-off value estimated for gal-1 that allows discrimination between poor and moderate differentiation was 13,708.29 pg/g. For this cut-off, we determined sensitivity to be 73.6% and specificity 60.0%.

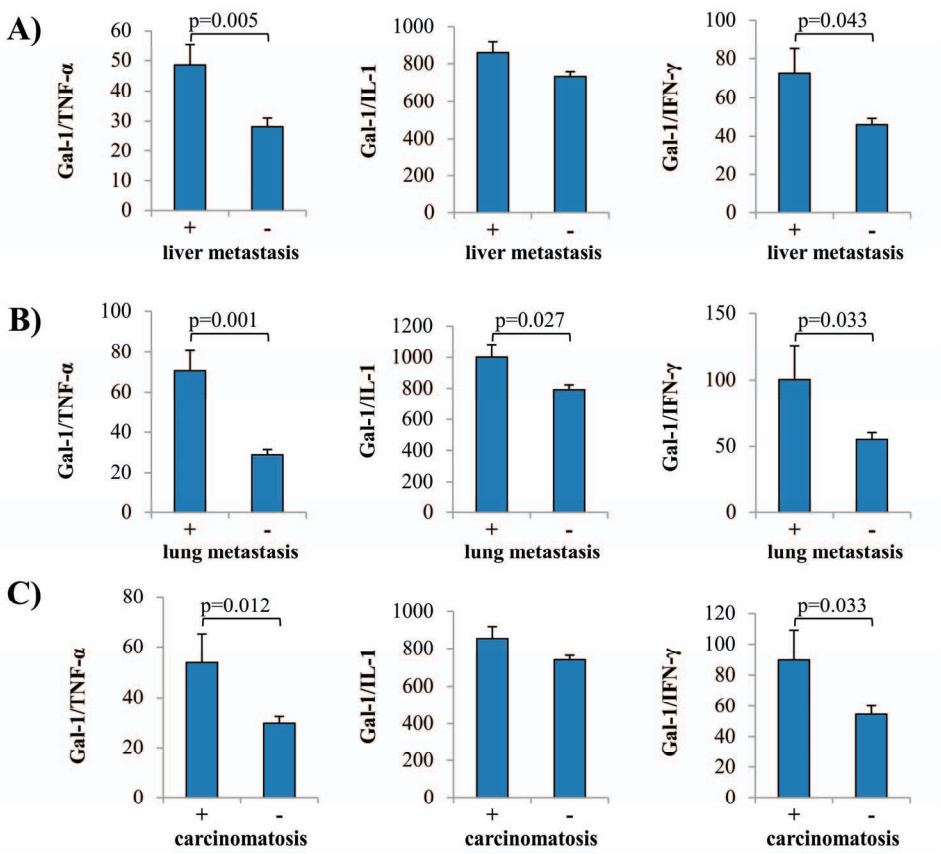

Fig. 3 – The systemic values of galectin-1 (gal-1)/tumor necrosis factor (TNF) α, gal-1/interleukin-1 (IL-1) and gal-1/interferon-gamma (IFN-γ) ratios in the patients with colorectal cancer (CRC), based on tumor progression.
A) The increased gal-1/TNF-α and gal-1/IFN-γ ratios in the patients with detectable liver metastasis. The patients with CRC were divided into two groups, based on the presence of liver metastasis (+ and -).
B) The increased Gal-1/TNF-α, Gal-1/IL-1 and Gal-1/IFN-γ ratios in the patients with detectable lung metastasis. The patients with CRC were divided into two groups, based on the presence of lung metastasis (+ and -).
C) The increased gal-1/TNF-α and gal-1/IFN-γ ratios in the patients with detectable peritoneal carcinomatosis. The patients with CRC were divided into two groups, based on the presence of carcinomatosis in peritoneum (+ and -).
The serum levels of all mentioned biomarkers were determined by enzyme linked immunosorbent assay (ELISA). The gal-1/TNF-α, gal-1/IL-1 and gal-1/IFN-γ ratios were evaluated for each patient, separately. A statistical significance was tested by the Mann–Whitney Rank Sum test, or the independent samples *t*-test, where appropriate.

Variables	Fecal Gal-1	
	Spearman`s rho	*p* value
Histological type	0,357	0.025
AFP	0,317	0.028
CEA	0,230	0.115
CA 19-9	0,296	0.049

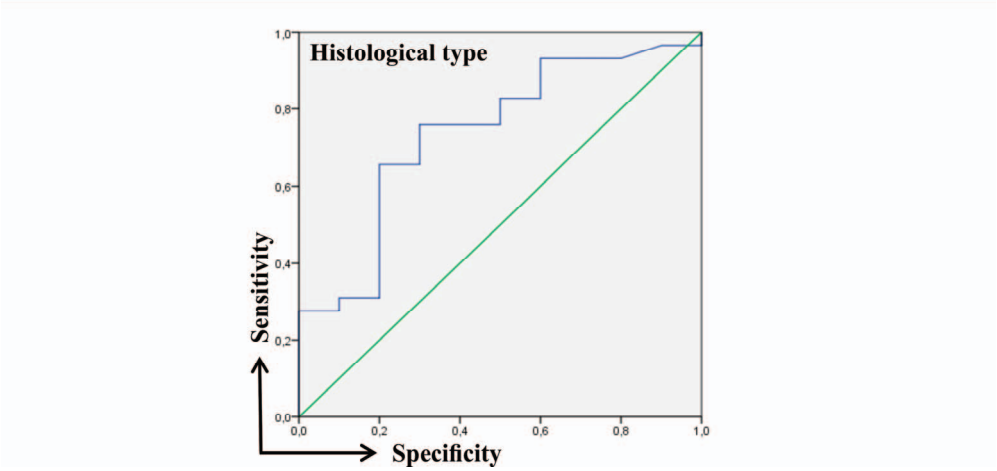

Fig. 4 – Fecal concentration of galectin-1 (gal-1) was positively associated with the poorly differentiated tumor and systemic values of tumor markers alpha-fetoprotein (AFP), carcino-embryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9), in the patients with colorectal cancer (CRC). The relationships between the values of gal-1 in feces and a histological differentiation stage of tumor tissue and concentrations of AFP, CEA and CA 19-9 in the serum were examined by the Spearman's correlation test. The receiver operations characteristic (ROC) curve illustrates the specificity and sensitivity of fecal gal-1 in an attempt to differentiate a histological differentiation stage of tumor tissue: well/moderate vs. poor differentiated.

Discussion

Biological role of gal-1 in the tumor cell proliferation, invasion, apoptosis, metastasis, immuno-suppression and tumor angiogenesis is well-known [20–27]. It is involved in a poor prognosis and the metastatic phenotype [23, 24]. Gal-1 may act intracellularly as well as extracellularly, after secretion [28]. Secreted gal-1 can interact with the cell-surface proteins such as fibronectin, integrins, laminin and vascular endothelial growth factor receptor 2 (VEGFR2) and subsequently determines the proliferation, adhesion, migration and angiogenesis [29, 30]. These findings highlight the importance of extracellular gal-1 in tumor biology. In the present study, we analyzed the systemic and fecal level of gal-1 and it`s ratio with several pro-inflammatory cytokines, in different stages of CRC. We found the increased concentration of gal-1 in the stool of CRC patients with a higher nuclear grade (III vs. II and III vs. I) and poor tumor tissue differentiation (Figure 1). Previous studies established gal-1 as a protein commonly elevated in the serum of patients with tumors [8–10]. Also, the serum gal-1 values were significantly increased in the patients with metastatic disease compared with the patients with localized tumors [11]. We did not find that the serum gal-1 mean values ranged significantly differently histopathological characteristics of tumor, while the fecal gal-1 showed a significant alteration according to the histopathological characteristics (Figure 1).

Indeed, in the recent studies, feces was used as a sample for testing different biomarkers [5, 6]. For instance, fecal calprotectin (FC), a biomarker of intestinal inflammation that has been in clinical use for years [5–7], also proved to be elevated in CRC and suggested for screening the high risk groups for CRC [31]. Today, the researchers test diagnostic accuracy of different fecal markers in the detection of cancerous lesions of the colorectum in order to find the most accurate one for CRC screening. According to the available literature, this is the first study testing fecal gal-1 for the detection of severe and progressive forms of CRC.

It was suggested that ratio of counterregulatory cytokines was a reliable marker of the disease progression [32]. Therefore, we considered the ratios of gal-1 and pro-inflammatory cytokines and showed the predomination of gal-1 over pro-inflammatory cytokines TNF-α, IL-1β and IFN-γ in the patients with CRC with progressive disease. The gal-1/TNF-α ratio in the serum and feces had a higher trend in the patients with the advanced TNM stage (III+IV) as well as detectable lymphatic and blood vessel invasion (Figure 2). In line with this finding, the enhanced gal-1/TNF-α and gal-1/IFN-γ ratios were detected in the serum of patients with presence of lung/liver metastasis or peritoneal carcinomatosis, while the enhanced gal-1/IL-1 ratio was detected only in the serum of patients with lung metastasis (Figure 3). Based on these findings, we believe that the gal-1/TNF-α ratio could be a predictor of the advanced stages of colorectal cancer.

The role of gal-1 in the onset, progression and resolution of inflammation is well-established [16]. Previous studies revealed that gal-1 inhibit cell growth and induce the apoptosis of activated immune cells [33, 34]. Gal-1 was shown to

skew the balance toward the type-2 immune response, simultaneously inhibiting IFNγ, TNFα, IL-2 and IL-12 production and facilitating IL-5 secretion, *in vitro* and *in vivo* [35–37]. Some studies suggest that gal-1 might inhibit T-cell effector functions, or induce the death of tumor infiltrating leukocytes and subsequently suppress a strong immune response derived by proinflammatory cytokines [14, 36, 38, 39]. We are first to describe prevailing of gal-1 over TNF-α, IL-1β and IFN-γ in the stool of patients with the severe and progressive forms of CRC (Figure 2 and 3). In line with our finding, Camby et al. [14] concluded that the tumor cells may impair the T-cell effector functions through the secretion of gal-1, that favors genesis of an immunosuppressive environment at a tumor site.

Further in this study, we envisage the possible role of fecal gal-1 as a biomarker in preceding disease severity. We found a positive correlation between the gal-1 value in feces and histological differentiation tumor of stage and biomarkers AFP and CA 19-9, respectively (Figure 4). Interestingly, we did not find a correlation of serum gal-1 with the same parameters and markers of disease severity. Also, the values of gal-1 in feces are about two to three times higher than in the serum, what makes measurement in feces a more sensitive method. The analysis of receiver operating characteristic (ROC) curves of gal-1 and the disease parameters and markers for CRC revealed that gal-1 could predict a poor differentiated type of tumor, at good sensitivity and specificity. According to our findings, fecal gal-1 could be a valuable marker for the CRC severity.

Conclusion

In summary, the increased local values of gal-1, reflected through a higher fecal concentration, in the CRC patients with a higher nuclear grade and poor tumor tissue differentiation may be considered as a sign of the tumor's malignant progression and, consequently, of a poor prognosis for the patients. The predominance of gal-1 over proinflammatory cytokines TNF-α, IL-1β and IFN-γ in the patients with advanced and progressive disease may implicate immunomodulatory role of gal-1 in limiting the ongoing proinflammatory processes and preventing a potent antitumor immune response. Furthermore, the fecal values of gal-1 can be used as a valuable marker for the CRC severity. These observations point to a possible role of fecal gal-1 as a state marker of CRC and its potential use as a therapeutic target.

Declaration of interest

The authors declare that they have no competing interests.

Acknowledgements

This work was supported by grants from the Serbian Ministry of Education, Science and Technological Development (175071, 175069 and 175103), and from the Faculty of Medical Sciences, Kragujevac, Serbia (project JP 04/15). The authors thank Milan Milojević and Aleksandar Ilić for excellent technical assistance.

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Received on December 11, 2017.
Revised on January 10, 2018.
Accepted on January 11, 2018.
Online First January, 2018.

ORIGINAL ARTICLE

UDC: 616.314-089.5
https://doi.org/10.2298/VSP170511013C

Efficacy and safety of 4% articaine with epinephrine for the anterior middle superior alveolar nerve block comparing to the computer-controlled and conventional anesthetic delivery: prospective, randomized, cross-over clinical study

Efikasnost i sigurnost 4% artikaina sa epinefrinom za gornji prednji i srednji alveolarni nervni blok primenom kompjuterski kontrolisanog sistema i standardnog pristupa za primenu anestetičkog rastvora: prospektivna, randomizovana, kontrolisana, dvosturko slepa, ukrštena klinička studija

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Abstract

Bacground/Aim. The efficient dental anesthesia, which is related to the clinically adequate depth, duration and the width of anesthetic field, is an important prerequisite for successful dental treatment. The aim of this study was to investigate and compare the pulpal anesthesia and cardiovascular parameters after the anterior middle superior alveolar (AMSA) nerve block with 4% articaine with epinephrine administered by conventional cartridge-syringe and computer-controlled local anesthetic delivery system (CCLADS). **Methods.** This controlled double-blind cross-over randomized clinical study included 38 healthy volunteers. Efficacy of pulpal anesthesia after the AMSA nerve block was evaluated by measuring a success rate, onset and duration of pulpal anesthesia, using an electrical pulp tester. The parameters of cardiovascular function (systolic and diastolic blood pressure, heart rate) were monitored noninvasively. **Results.** Successful pulpal anesthesia of all tested teeth was obtained in 57.9% participants with CCLADS and in 44.7% participants with conventional syringe. The onset time was not significantly different between two investigated groups. The pulpal anesthesia duration was not significantly different neither within nor between investigated groups. The systolic and diastolic blood pressures were significantly decreased in both investigated groups, in comparison with the baseline values. Heart rate significantly decreased within CCLADS from 10th to 30th minute when compared to baseline. **Conclusion.** The efficacy of pulpal anesthesia and safety of cardiovascular profile of 0.6 mL of articaine with epinephrine (1:100.000) delivered with CCLADS were improved in comparison to the conventional syringe delivery. Significant changes of cardiovascular function were not observed.

Key words:
articaine; anesthesia dental; anesthesia, local; therapy, computer assisted; nerve block; injections.

Apstrakt

Uvod/Cilj. Efikasna zubna anestezija u pogledu klinički adekvatne dubine, trajanja i širine anestetičkog polja je važan preduslov za uspešno zubno lečenje. Cilj rada bio je ispitivanje i upoređivanje parametra anestezije zubne pulpe i kardiovaskularnih parametra posle gornje prednje i srednje alveolarne (AMSA) sprovodne anestezije postignute 4% artikainom sa epinefrinom, primenom standardne karpul-brizgalice i kompjuterski kontrolisanog sistema za primenu anestetičkog rastvora (CCLADS). **Metode.** U ovom randomizovanom, prospektivnom, kontrolisanom, dvostruko slepom ukrštenom kliničkom istraživanju učestvovalo je 38 ispitanika. Kvalitet anestezije zubne pulpe posle AMSA anestezije praćen je na osnovu uspešnosti, latentnog perioda i trajanja anestezije zubne pulpe, primenom električnog pulp-testera. Parametri kardiovaskularne funkcije (sistolni i dijastolni krvni pritisak, srčana frekvencija) praćeni su neinvazivno, primenom aparata za monitoring. **Rezultati.** Uspešna anestezija zubne pulpe svih ispitivanih zuba bila je

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prisutna kod 57,9% ispitanika posle kod CCLADS i kod 44,7% ispitanika posle primene standardne karpul-brizgalice. Latentni period i trajanje anestezije zubne pulpe nisu se značajno razlikovali između ispitivanih grupa. Sistolni i dijastolni pritisak bili su značajno sniženi u praćenim vremenskim intervalima u odnosu na početne vrednosti. Srčana frekvencija je bila značajno snižena kod CCLADS grupe od 10 do 30 minuta u odnosu na početne vrednosti. **Zaključak.** Kontrolisanom kompjuterizovanom primenom (CCLADS) 0,6 mL 4% artikaina sa epinefrinom (1:100,000) za AMSA sprovodnu anesteziju, postignut je bolji kvalitet anestezije zubne pulpe u odnosu na primenu artikaina sa epinefrinom standardnom karpul brizgalicom. Nisu uočene bitne promene funkcija kardiovaskularnog sistema.

Ključne reči:
artikain; anestezija, stomatološka; anestezija, lokalna; lečenje, kompjuterom vođeno; blokada živca; injekcije.

Introduction

The efficient dental anesthesia, which is related to the clinically adequate depth, duration and the width of anesthetic field, is an important prerequisite for successful dental treatment. Most commonly, the pulp of the maxillary teeth, surrounding bone and soft tissue are anesthetized by buccal supraperiosteal infiltration of local anesthetic in the projection of the tooth apex [1]. Recently, the anterior middle superior alveolar (AMSA) nerve block has been introduced as a technique which provides pulpal anesthesia of multiple maxillary teeth with single site injection, without collateral anesthesia of lip, face and muscles of facial expression [2]. The local anesthetic solution for the AMSA nerve block is deposited palatal, at the point which bisects the maxillary premolars, and midway between the mid-palatal raphe and premolar free gingival margin crest. The target area is the anatomical region where the anterior superior alveolar (ASA) and the medial superior alveolar (MSA) nerves' branches converge and form the dental neural plexus. Therefore, an anesthetic effect of a single AMSA block injection extends to the pulpal tissue of maxillary teeth from the central incisor through the second premolar, innervated by the ASA and MSA nerves, with additional anesthesia of soft palatal tissue in the same region.

Generally, the palatal injections with a conventional syringe have the potential to be unpleasant and painful. The new approach in the dental anesthesia techniques develops systems of controlled anesthetic delivery is aimed to reduce not only a patient's discomfort during palatal soft tissue anesthesia, but also to increase quality of bone and dental pulp anesthesia. Some of the published studies using this computer-controlled local anesthetic delivery system (CCLADS) for the AMSA nerve block, showed that continuous and controlled dosage of 2% lidocaine with epinephrine may achieve profound pulpal anesthesia from central incisor through second premolar, what was not seen after conventional delivery [2–4]. Although obtained clinical results were more favorable with the CCLADS than with conventional delivery, the modest to low success rate, slow onset and rapid anesthesia duration declining observed after 2% lidocaine with epinephrine may decrease the clinical utilization of AMSA nerve block due to the anatomical properties of palatal injection site, as well as pharmacological properties of lidocaine with epinephrine solution [5, 6].

Since the AMSA nerve block is obtained following diffusion of local anesthetic solution through the palatal bone before reaching the dental nerve plexus, the characteristics of local anesthetic could influence the parameters of local anesthesia. Articaine is an amide local anesthetic with the unique properties due to the characteristics of thiophene ring in its structure, which ensures that a diffusing property of articaine through soft and hard tissues is more pronounced than of other amide local anesthetics [7]. There is evidence that palatal soft tissue anesthesia occasionally may be obtained by articaine diffusion after maxillary infiltration without an additional palatal injection [8–10].

To our knowledge, there are no data concerning the efficacy and safety of AMSA nerve block obtained with 4% articaine with epinephrine. Therefore, the aim of this study was to investigate and compare the parameters of pulpal anesthesia and cardiovascular functions after the AMSA nerve block with 4% articaine with epinephrine administered by a conventional syringe and the CCLADS.

Methods

This prospective, double-blind cross-over randomized study included the healthy volunteers [American Socity of Anesthesiologists (ASA) I] who were admitted for the regular dental examination at the Clinic of Oral Surgery, School of Dental Medicine, University of Belgrade, Serbia. After approval of the Ethics committee of the School of Dental Medicine (36/5-2015), the study was performed in accordance with ethical standards laid down in 1964 Declaration of Helsinki and its later amendments. The United States National Institutes of Health Clinical Trial registration was performed (ClinicalTrials.gov: NCT02440347). All participants signed the informed consent after they were informed in details, verbally and in written form, about the study procedures and possible side effects.

Participants' eligibility

Inclusion criteria were as follows: full maxillary dental arch with all maxillary teeth free of caries and restorations, without history of trauma, sensitivity or orthodontic treatment, with a visible pulpal chamber on intraoral radiogram, responsive to electrical stimulation by the pulp tester and clinically and radiographically healthy periodontium. The allergy to local anesthetics, presence of orofacial pain, and/or use of any medicine within the previous 48 hours, pregnancy, lactation, drug and/or alcohol abuse, tobacco smoking and inhibition to give informed consent were exclusion criteria.

Study procedure

The patients served as their own control in cross-over design. The AMSA injection on one side of the maxillary arch was administered using CCLADS (Anaeject®, Septodont, France) while conventional cartridge-syringe was used for the opposite side. The AMSA injection site was located at a point that bisects the maxillary first and second premolars, and midway between the crest of the free gingival margin and mid-palatine suture. The needle was orientated at a 45 degree angle with the bevel facing palatal tissue. All dental anesthesia glass cartridges were marked at the level of 0.6 mL of anesthetic solution. The 0.6 mL of 4% articaine with epinephrine 1:100 000 was administered in two minutes period, both with CCLADS, or conventional cartridge-syringe. CCLADS enabled a slow and constant administration of anesthetic, approximately 0.005 mL per second. The sound mode during the administration of local anesthetic with CCLADS was switched off, in order to disable the patient to distinguish the method of administration. The patients were instructed to rest prior to the injection in supine position for 15 minutes in an isolated dental office. In addition, the patients were blindfolded with a commonly used sleeping mask so they would not distinguish which anesthetic delivery system was used. The same operator administered anesthesia of both sides and was excluded from measuring data and statistical analysis. The order of anesthesia techniques was blinded and randomly selected from a sealed envelope for each patient, with a washout period of 2 weeks between the appointments.

Parameters of pulpal anesthesia

The pulpal anesthesia parameters were tested in the following teeth: upper central and lateral incisor, canine, first premolar and second premolar with contralateral canine as a control tooth.

The anesthetic success, onset and duration were registered with the electrical pulp tester (EPT) (Vitality Scanner, Sybron Endo Model 2006®, Orange, CA, USA). The primary outcome was a success of pulpal anesthesia. The anesthesia was considered successful when 2 consecutive no response at maximum (80) readings stimulation were obtained. Before the anesthesia was given, the experimental teeth and contralateral control canine were tested by means of Vitality Scanner to record the baseline vitality. The fluoride gel (Fluorogal Forte®, Galenika, Belgrade, Serbia) was used as an electrolyte between the tooth and pulp tester probe. The tip of pulp tester probe was placed in the middle third of the buccal side of tested teeth. The pulp response was tested immediately after the end of anesthetic application, from the central incisor through second premolar order, making the repeated measurements for each tooth 4 minutes apart.

The secondary outcomes were onset and duration of pulpal anesthesia. Onset time for anesthesia was defined as the time from completion of the anesthetic injection to the time when profound anesthesia was achieved EPT ≥ 80. The duration of pulpal anesthesia was a period between the first and the last 80 readings on EPT, what was considered to be profound anesthesia.

Parameters of cardiovascular function

The cardiovascular parameters, such as the systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were recorded by the electrocardiogram monitor (Datex-Engstrom AS/3, Helsinki, Finland) 6 times: 5 minutes prior anesthesia, during anesthetic injection, and 5, 10, 15 and 30 minutes after administering anesthesia. Also, the participants were monitored for the potential changes in a cardiac rhythm and the possible signs of myocardial ischemia.

Statistical analysis

The data concerning the demographic characteristics of participants were compared by the χ^2-test. A success of pulpal anesthesia was evaluated by the descriptive statistical methods with the χ^2-test and Fisher's test for within group analysis and the McNemar test for between groups comparisons. The obtained differences in the onset and duration of pulpal anesthesia between the groups were tested for a significance by the Wilcoxon Sign Rank test, while the Kruskall-Wallis test was used for within group analysis, followed by the Mann-Whitney *U* test. The parameters of cardiovascular function (SBP, DBP and HR) were analyzed by the two-way repeated measures ANOVA with the group and time as the main factors. The changes of cardiovascular parameters over time within investigated groups (baseline vs. observed time intervals), were compared by means of one-way repeated measures ANOVA with the Bonferroni *post hoc*. Between group comparisons in the cardiovascular function at the observed time intervals were performed by the paired samples *t*-test. The statistical analysis was carried out using the SPSS 20.0 (Inc., Chicago, IL, USA) with the level of significance set at 0.05. It was necessary to include at least 34 participants to obtain 20% difference in the pulpal anesthesia duration between the techniques with 80% statistical power at a two-tailed level of significance 0.05 using the Mann Whitney *U* test.

Results

Demographic data and participants flow

The participants flow is presented in Figure 1. Sixty-seven participants were assessed for eligibility, and 40 of them were recruited to participate in this study. All participants received both treatments, but two of them had unsuccessful anesthesia with both delivery systems and were excluded from the further analysis. The final number of analyzed participants with successful pulpal anesthesia of at least one tested tooth was 38, 20 females and 18 males, aging between 19 and 31 years (25.27 ± 2.46) with the mean body mass of 67.30 ± 15.03 kg/m^2.

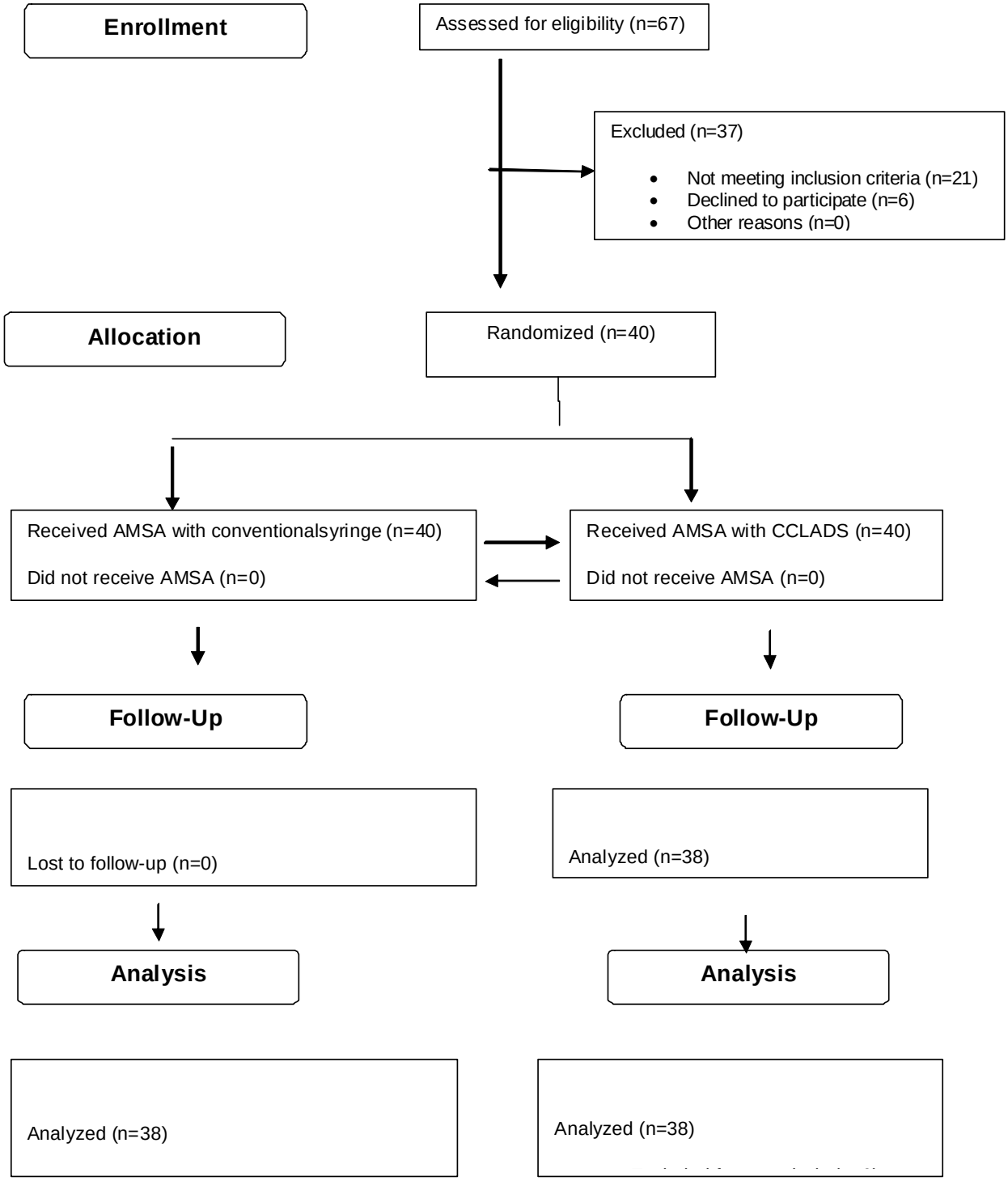

Fig. 1 – Flow diagram of randomization to either conventional injection, or computer-controlled local anesthetic delivery system (CCLADS) for the anterior and middle superior alveolar (AMSA) nerve block (CONSORT Statement 2010 Flow Diagram from Schulz KF, Altman DG, Moher D, for the CONSORT Group (2010) CONSORT 2010 statement: Updated guidelines for parallel group randomized trials. J Pharmacol Pharmacother. doi: 10.4103/0976-500X.72352).

Parameters of pulpal anesthesia

Successful pulpal anesthesia of all tested teeth was achieved in 22 (57.9%) participants of the CCLADS group and in 17 (44.7%) participants of the conventional syringe group. A success rate with CCLADS was 68.4% for the central incisor, 100% for the lateral incisor and canine, 76.3% for the first premolar and 86.8% for the second premolar; with the conventional syringe, a successful anesthesia was achieved in 71% for the central incisor, 94.7% for the lateral

incisor, 81.6% for the canine, 71% for the first premolar and 68.4% for the second premolar. Within group analysis of pulpal anesthesia a success rate in the CCLADS group revealed: a significantly higher success for the lateral incisor in comparison with the central incisor ($p < 0.001$) as well as for the lateral incisor in comparison with the first premolar ($p = 0.012$), and also for the canine in comparison with the central incisor ($p < 0.001$), and for the canine compared to the first premolar ($p = 0.012$). In the conventional injection group, pulpal anesthesia was significantly more successful for the lateral incisor compared to the central incisor ($p = 0.012$), for the lateral incisor compared to the first premolar ($p = 0.012$), as well as for the lateral incisor compared to the second premolar ($p = 0.006$). Between groups comparison showed that for the canine and second premolar a significantly higher success rate of pulpal anesthesia was observed after CCLADS vs. conventional injection (Table 1).

The onset and duration of pulpal anesthesia with conventional syringe and CCLADS are presented in Table 2. The onset time was not significantly different between these 2 investigated anesthetic techniques. Within group comparison revealed that there were no significant differences in the onset time among the teeth after the CCLADS injection. However, the onset of anesthesia was significantly longer in the conventional injection group for the central incisor in comparison with the lateral incisor ($p = 0.024$), for the central incisor compared to the first premolar ($p = 0.037$), and also when the central incisor was compared to the second premolar ($p = 0.022$), as well as for the canine in comparison with the first premolar ($p = 0.044$). The significant differences in the pulpal anesthesia duration were not observed neither within nor between the investigated groups (Table 2).

Table 1

Success of pulpal anesthesia after anterior middle superior alveolar (AMSA) injection of 4% articaine with conventional syringe and the computer-controlled local anesthetic delivery system (CCLADS)

Tooth	Delivery system		
	Conventional syringe (n/N)	CCLADS (n/N)	p^a
Central incisor	27/38	26/38	ns
Lateral incisor	36/38*	38/38*	ns
Canine	31/38	38/38*	0.016
First premolar	27/38	29/38	ns
Second premolar	26/38	33/38*	0.016
p^b	0.020	< 0.001	

N – number of participants; n – number of successfully anesthetized teeth.
[a] – between groups comparison (McNemar test); [b] – within group comparisons (χ^2-test, Fisher's test *post hoc*); *$p < 0.05$: conventional injection for the lateral incisor vs. the central incisor ($p = 0.012$), the lateral incisor vs. the first premolar ($p = 0.012$), the lateral incisor vs. the second premolar ($p = 0.006$); the CCLADS injection for the lateral incisor vs. the central incisor ($p < 0.001$), the lateral incisor vs. the first premolar ($p = 0.012$), the canine vs. the central incisor ($p < 0.001$), the canine vs. the first premolar ($p = 0.012$).

Table 2

Onset and duration of successful pulpal anesthesia after the anterior middle superior alveolar (AMSA) injection of 4% articaine with a conventional syringe and the computer-controlled local anesthetic delivery system (CCLADS)

Tooth	Onset			Duration		
	Conventional (n)	CCLADS (n)	p^a	Conventional (n)	CCLADS (n)	p^a
Central incisor	10.61 ± 5.31* (27)	(26)	0.692	24.62 ± 18.92 (27)	28.31±15.79 (26)	0.411
Lateral incisor	8.01 ± 4.17 (36)	8.00 ± 4.68 (38)	0.959	33.14 ± 19.95 (36)	28.11 ± 17.51 (38)	0.168
Canine	9.45 ± 4.58* (31)	8.68 ± 5.03 (38)	0.149	32.19 ± 17.04 (31)	29.58 ± 19.38 (38)	0.710
First premolar	7.07 ± 5.45 (27)	7.74 ± 6.59 (29)	0.646	32.55 ± 14.30 (27)	26.34 ± 9.26 (29)	0.057
Second premolar	7.08 ± 5.05 (26)	6.84 ± 5.02 (33)	0.896	31.62 ± 16.11 (26)	29.46 ± 17.94 (33)	0.407
p^b	0.042	0.172		0.251	0.821	

Values given in minutes as mean ± standard deviation (SD); n – number of successfully anesthetized teeth.
[a]between groups comparison (Wilcoxon Sign Rank test); [b] within group comparison (Kruskal-Wallis test, Mann-Whitney *U* test *post hoc*); *$p < 0.05$: conventional injection for the central incisor vs. the lateral incisor ($p = 0.024$), the central incisor vs. the first premolar ($p = 0.037$), the central incisor vs. the second premolar ($p = 0.022$); the canine vs. the first premolar ($p = 0.044$).

Parameters of cardiovascular function

The two-way repeated measures ANOVA revealed a significant effect of time factor for SBP ($p < 0.001$), DBP ($p < 0.001$) and HR ($p = 0.008$), with the significant interaction of main factors only for SBP ($p = 0.010$) (data not presented).

The repeated measures one-way ANOVA with the Bonferroni *post hoc* test in the CCLADS group showed a significant decrease in SBP 5 minutes ($p < 0.001$), 10 minutes ($p = 0.009$) and 15 minutes ($p = 0.008$) after an injection in comparison with the baseline values; after conventional injection, SBP was significantly lower after 10 minutes ($p = 0.001$), 15 minutes ($p = 0.001$) and 30 minutes ($p = 0.029$) when compared to the baseline values. Significantly lower SBP was observed at 5th minute ($p = 0.006$) after conventional vs. CCLADS injection (Figure 2).

The statistical analysis revealed that there was a significant decrease in DBP over time in the CCLADS group at 5th minute ($p = 0.037$) and 10th minute ($p = 0.036$) when compared to the baseline values, while after conventional injection, a significant decrease in DBP was observed after 10 minutes ($p = 0.001$) and 15 minutes ($p = 0.006$) in comparison with the baseline values (Figure 3).

The significant changes in HR were observed only in the CCLADS group during the observation time, with the significantly decreased values at 10th ($p = 0.008$), 15th ($p = 0.008$) and 30th minute ($p = 0.003$) when compared to the baseline (Figure 4).

The changes in the cardiac rhythm and signs of myocardial ischemia were not observed on electrocardiogram during the observation period.

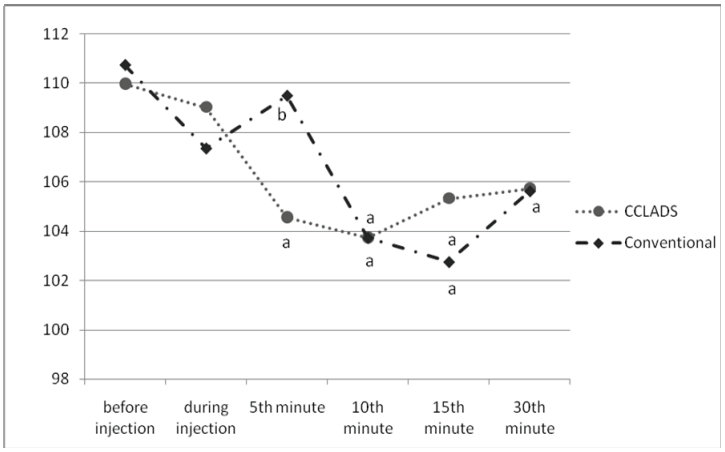

Fig. 2 – Changes in systolic blood pressure.

[a] within group comparison, One-way repeated measures ANOVA (baseline vs. observed time interval, Bonferroni *post hoc* test); for CCLADS 5th min ($p < 0.001$), 10th min ($p = 0.009$), 15th min ($p = 0.008$); for conventional injection 10th min ($p = 0.001$), 15th min ($p < 0.001$), 30th min ($p = 0.029$).
[b] between groups comparison, Paired samples *t*-test, 5th min ($p = 0.006$).
CCLADS – computer controlled local anesthetic delivery system.

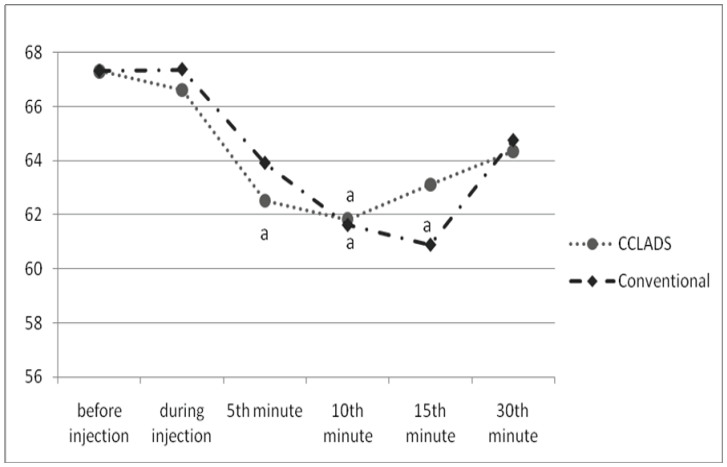

Fig. – 3 Changes in diastolic blood pressure.

[a] within group comparison; One-way repeated measures ANOVA (baseline vs. observed time interval, Bonferroni *post hoc* test); for CCLADS 5th min ($p = 0.037$), 10th min ($p = 0.036$); for conventional injection 10th min ($p = 0.001$), 15th min ($p = 0.006$).
CCLADS – computer controlled local anesthetic delivery system.

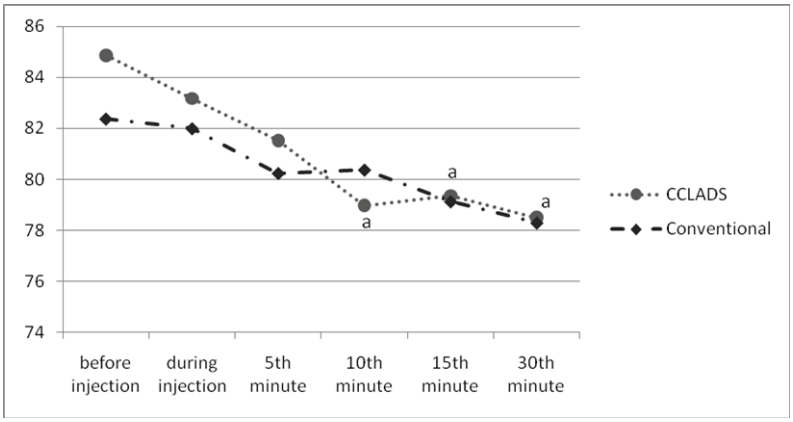

Fig. 4 – Changes in heart rate.

[a] within group comparison, One-way repeated measures ANOVA (baseline vs. observed time interval, Bonferroni *post hoc* test); for the CCLADS 10th min ($p = 0.008$), 15th min ($p = 0.003$), 30th min ($p = 0.003$).
CCLADS – computer controlled local anesthetic delivery system.

Discussion

This prospective, controlled double-blind, cross-over randomized clinical study in the healthy volunteers evaluated and compared the anesthetic and cardiovascular effects of 0.6 mL 4% articaine with epinephrine (1:100.000) used for the AMSA nerve block delivered by the computer-controlled delivery system and conventional syringe.

The overall success rate of pulpal anesthesia, as a primary outcome of the study, with CCLADS ranged from 68.4% to 100%, while with conventional syringe, it was between 68.4% and 94.7%. Pulpal anesthesia in the present study was considered successful if two, or more consecutive 80 readings were obtained by the electrical pulp test without the participant's response. Regarding the fact that the parameters of pulpal anesthesia after the AMSA nerve block obtained with articaine with epinephrine were not investigated, the available evidence concern the use of 2% lidocaine with epinephrine as a "gold standard". Namely, in the study of Fukayama et al. [3], the lower success rates were observed (between 42% and 72%), based on one registered 80 reading without a participant's response during the electrical stimulation, after the AMSA nerve block obtained with 1.8 mL of 2% lidocaine with epinephrine 1 : 80.000 delivered by the computer controlled system WAND. Similarly low success rates, evaluated according to two consecutive 80 readings without a participant's response to the electric pulp test, were also reported in a comparative study by Lee et al. [5] during the 60-minute observation period, after 1.4 mL of 2% lidocaine with 1 : 100.000 epinephrine delivered for the AMSA nerve block, either by computer controlled system (success rate between 35% and 58%), or using conventional syringe (success rate between 20% and 42%). On the other hand, Corbett et al. [6] reported a higher anesthetic success of pulpal anesthesia (between 42% and 85%) during the 47-minute observation period, regarding minimum two consecutive 80 readings without a response to the electrical stimuli, when 1 mL of 2% lidocaine with epinephrine 1 : 80.000 was delivered with the WAND system for the AMSA nerve block in comparison with the infraorbital nerve block. Considering factors that influence a success rate of pulpal anesthesia, as an important parameter of anesthesia quality, several of them, such as the anesthetic pharmacological profile, regional anatomical specificity and local anesthesia technique, could contribute to a higher success rate of local intraoral anesthesia. Since no difference was observed between delivery systems (CCLADS and conventional cartridge-syringe), our results could be explained rather by pharmacological properties of local anesthetic applied than the anesthetic technique used. Articaine is an amide local anesthetic with the characteristic molecular structure that includes the presence of tiophene ring. The presence of tiophene ring increases the anesthetic liposolubility, and it is suggested that articaine diffuses more readily through the soft and bone tissue than other local anesthetics. Increased lipid solubility of articaine (partition coefficient 17) in comparison with lidocaine (partition coefficient 4) permits the more active anesthetic molecules to effectively penetrate the lipid nerve membrane, what is reflected in the increased anesthetic potency of articaine. Concerning that, it is clear that articaine provides higher rates of pulpal anesthesia success than lidocaine when used for infiltration and block anesthesia during the routine dental treatments [11].

Further analysis of success rate in the present study compared the efficacy of CCLADS and conventional syringe delivery in obtaining successful pulpal anesthesia of specific teeth. Our results showed that pulpal anesthesia of canine and second premolar was significantly more successful after CCLADS injection in comparison with conventional injection. A significantly higher success rate of pulpal anesthesia using CCLADS compared to conventional injection was also observed in a comparative study by Lee et al. [5], but for all tested teeth from the lateral incisor to the second premolar after the AMSA nerve block achieved with 1.4 mL of 2% lidocaine with epinephrine 1 : 100.000. A lower success rate obtained with conventional injection could be due to the difference in the pressure gradient and flow rate between computer controlled and conventional delivery, since, using computer controlled delivery system, both factors were more precisely controlled and constant, providing the better conditions for anesthetic solution diffusion through the alveolar

and palatal bone. Furthermore, within the groups, the most successful pulpal anesthesia was observed for the lateral incisor and canine, while the lowest success rate was observed for the central incisor and first premolar regardless the delivery system used. The obtained results could be explained by the anatomical variability in innervation of the anesthetized area, since it is possible that the buccal root of the first premolar may be innervated by the accessory branches of posterior superior alveolar nerve [12]. Also, the buccal position of the root defines the greater distance and diffusion path of anesthetic solution which is deposited palatal. Similarly, the distance of central incisor's root from the deposition site could affect diffusion of anesthetic solution, leading to a comparatively lower pulpal anesthesia success rate.

The onset of pulpal anesthesia was similar between the investigated groups in our study. The results of previous studies, after the AMSA nerve block performed with 2% lidocaine with epinephrine, showed that the onset time ranged between 5 and 11 minutes with CCLADS delivery system, and between 6 and 12 minutes with conventional injection delivery [4, 6]. Similar results were obtained in our study. Similarly to the previously mentioned studies, the longer onset time was observed for the central incisor and canine, using both delivery systems. It is possible that this delay in the onset was due to a greater diffusion distance of anesthetic solution through the alveolar and palatal bone [6].

Only two studies reported that the duration of pulpal anesthesia after the AMSA nerve block was achieved with 2% lidocaine with epinephrine. In a study by Fukayama et al. [3], the authors reported the duration of pulpal anesthesia up to 40 minutes, including all tested teeth from the central incisor to the first molar, after the AMSA nerve block obtained with 2% lidocaine with epinephrine 1:80.000 using CCLADS delivery system. Similarly, Velasco and Soto [4] observed the duration of pulpal anesthesia of tested teeth between 23 and 40 minutes when 2% lidocaine with epinephrine 1:100.000 was applied with the conventional cartridge-syringe [4]. However, in the mentioned studies, the statistical analysis of pulpal anesthesia duration was not performed due to a low number of participants with a successful pulpal anesthesia. In the present controlled clinical study, with confirmed statistical power, duration of pulpal anesthesia comparing two delivery systems was similar and ranged from 24 to 32 minutes for conventional syringe delivery, and between 26 to 29 minutes for CCLADS. Regarding the pharmacological profile of articaine, the degree of articaine protein binding (95%), which is higher than that of lidocaine (65%), is expected to ensure the more firm attachment of articaine molecules to the protein receptors sites, responsible for a longer duration of clinical activity. However, further comparative study of articaine and lidocaine might confirm anesthetic superiority concerning anesthesia duration after articaine delivery for the AMSA nerve block.

It still remains unclear whether the AMSA nerve block truly acts as a nerve block, since anesthetic solution is not deposited directly in the vicinity of any main nerve trunk. Furthermore, a success as well as duration of pulpal anesthesia varied with the distance and bone thickness between the tooth apex and deposition site, which favors more tissue infiltration as a type of anesthesia, over the nerve block conduction [6]. Taking all together, there could be a possibility that the AMSA nerve block acts as an intraosseous injection, which directly reflects to a significance of articaine + epinephrine safety profile. This route of administration, due to the deposition of local anesthetic in the highly vascularized bone area, allows the rapid resorption of both, local anesthetic and vasoconstrictor. Therefore, the safety profile of AMSA nerve block in the present study was evaluated by the cardiovascular parameters monitoring. The systolic blood pressure was a significantly decreased within CCLADS group from 5th to 15th minutes in comparison with the baseline values, and in the conventional group, from 10th to 30th minute when compared to the baseline. At the 5th minute, the systolic blood pressure significantly increased after the conventional injection in comparison with CCLADS. The decreased systolic blood pressure within both groups in our study implies a good safety of AMSA technique with regard to cardiovascular stability, which is supported also by the decreased values of diastolic blood pressure from 5th to 10th minute for the CCLADS injection and from 10th to 15th minute for conventional injection, in comparison with the baseline values. A heart rate was significantly decreased only in the CCLADS group from 10th to 30th minutes, which suggests the more preferable cardiovascular profile after CCLADS injection, since the heart rate is a cardiovascular parameter that is the most sensitive to the exogenous epinephrine effects [13].

Conclusion

In conditions of the present double-blind randomized controlled cross-over clinical study, the efficacy of pulpal anesthesia, especially for the lateral incisor and canine as well as safety of cardiovascular profile of 0.6 mL of articaine with epinephrine (1:100.000) delivered using CCLADS overcome conventional syringe delivery.

Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants No. 175021 and 175061) and the Secretariat for Higher Education and Scientific Research of the Autonomic Province of Vojvodina, Serbia (Project: Influence of adding strontium carbonate and strontium fluoride on physico-chemical and biologic characteristics of calcium silicate dental cements).

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Received on May 11, 2017.
Revised on January 10, 2018.
Accepted on January 11, 2018.
Online First January, 2018.

ORIGINAL ARTICLE

UDC: 001.891.5:[37.018.262:616-085
https://doi.org/10.2298/VSP170721002U

Pharmacotherapy literacy questionnaire for parents of pre-school children in Serbia: construction and psychometric characteristics

Upitnik za procenu farmakoterapijske pismenosti roditelja predškolske dece u Srbiji: konstrukcija i psihometrijske karakteristike

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Abstract

Background/Aim. Valid and reliable instruments were emphasized in the studies of pharmacotherapy literacy which is the capacity to obtain, evaluate, calculate, and comprehend basic information about pharmacotherapy and actions necessary to make appropriate medication-related decisions. The aims of this study were: to develop an instrument for assessment of pharmacotherapy health literacy among parents of pre-school children in Serbia (PTHL-SR) and to evaluate psychometric properties. **Methods.** This study was a four-stage methodological one, conducted from November 2015 to October 2016. The instrument content was established through qualitative and quantitative expert reviews in the first and second phase. Experts had to answer about the clarity and relevance of questions. The Content Validity Ratio (CVR) and index (CVI) were calculated based on the necessity and relevance of questions. Third phase was pre-testing of initial instrument to assess comprehensibility of questions. In the fourth phase, 300 parents completed questionnaire at several kindergartens in Belgrade, to determine questionnaire's reliability through internal consistency, using the Cronbach's alpha coefficient and correlation between classes. **Results.** The 14-items questionnaire was developed (initial PTHL-SR) and pre-tested on a pilot sample. It had 4 groups of questions about knowledge, understanding, numerical skills and access to medicines-related information. The Content Validity Ratio (CVR = 0.875) was significant and adequate (Lawshe CVR_s = 0.780). **Conclusion.** PTHL-SR is a reliable instrument for assessment of pharmacotherapy literacy among parents of pre-school children in Serbia and can be used for the evaluation of understanding, calculating and accessing medicines-related information.

Key words:
drug therapy; parents; child, preschool; knowledge; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Validni i pouzdani instrumenti neophodni su u studijama farmakoterapijske pismenosti koja predstavlja sposobnost dobijanja, procene, izračunavanja i razumevanja osnovnih informacija o farmakoterapiji i radnjama potrebnim za donošenje adekvatnih odluka koje se odnose na primenu lekova. Ciljevi ove studije bili su: da se razvije instrument za procenu farmakoterapijske pismenosti roditelja predškolske dece u Srbiji (PTHL-SR) i da se procene psihometrijske karakteristike ovog instrumenta. **Metode.** Studija je bila metodološka, u četiri faze, sprovedena od novembra 2015. do oktobra 2016. Sadržaj instrumenta napravljen je kroz kvalitativne i kvantitativne ocene eksperata u prve dve faze. Eksperti su trebali da ocene jasnoću i relevantnost pitanja. Odnos sadržajne validnosti (CVR) i indeks (CVI) izračunati su na osnovu relevantnosti i potrebe za pitanjima. Treća faza bila je pretestiranje inicijalnog instrumenta sa ciljem da se ustanovi razumljivost pitanja. U četvrtoj fazi, 300 roditelja popunilo je upitnik u nekoliko vrtića u Beogradu, da bi se odredila pouzdanost upitnika kroz internu i eksternu konzistentnost, korišćenjem Kronbah-alfa koeficijenta i korelacije između klasa. **Rezultati.** Konstruisan je upitnik sa 14 pitanja (inicijalni PTHL-SR) i izvršeno je pretestiranje na pilot uzorku. Upitnik sadrži 4 grupe pitanja: o znanju, razumevanju, numeričkim veštinama i pristupu informacijama o lekovima. Odnos sadržajne validnosti (CVR = 0.875) bio je značajan i odgovarajući (Lawshe CVR_s = 0.780). **Zaključak.** PTHL-SR je pouzdan instrument za procenu farmakoterapijske pismenosti roditelja predškolske dece u Srbiji i može se upotrebiti za procenu razumevanja, numeričkih veština i pristupa informacija o lekovima.

Ključne reči:
lečenje lekovima; roditelji; deca, predškolska; znanje; ankete i upitnici.

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Introduction

According to the definition of the American Medical Association (AMA), health literacy is "the constellation of skills, including the ability to perform basic reading and numerical skills to function in the health care environment" [1].

It is one of the most important social determinants of health which includes competences such as the critical and interactive skills as well as the numerical skills [2]. A person with adequate health literacy is the one who puts its own health and health of his/her family in an appropriate context, understands which factors can affect the health and knows to use health-related information [3, 4].

It was found that the parents with low health literacy harder understand the importance of vaccination and are not completely able to realize the risks of malnutrition and overweight. They can hardly recognize hazards in the home, which leads to frequent injuries of children [4, 5–8].

The parents with low health literacy often have difficulties to understand information given by a doctor as well as information on the baby food and medicine labels. Written information about medicines use are too complex for the parents with low health literacy [4, 5]. Furthermore, low health literacy among the parents cause difficulties to dose the medicine which is purchased without a prescription (OTC) for a child. Errors in dosing the OTC medicines to children can cause the risk of adverse effects and therapeutic failure [6, 7].

According to study conducted in France, oral antibiotic medicines were incorrectly reconstituted by the parents and caregivers in about 50% cases, with a risk of overdosing or underdosing. It was also noted that it is necessary to check if the parents understand the instructions about the use of oral liquid medications for children [9].

The level of health literacy may have a significant impact on the way healthcare professionals interact and communicate with the parents of children [10].

There is an association between low health literacy and misunderstanding of information related to medicines [6, 7], and health literacy related to use of medicines, i.e., "pharmacotherapy literacy" is „An individual's capacity to obtain, evaluate, calculate, and comprehend basic information about pharmacotherapy and pharmacy related services necessary to make appropriate medication-related decisions, regardless of the mode of content delivery (e.g., written, oral, visual images and symbols)" [11].

There are several general instruments intended to measure the level of health literacy [the Rapid Estimate of Adult Literacy in Medicine (REALM), the Short Test of Functional Health Literacy in Adults (S-TOFHLA), the Newest Vital Sign (NVS), the Wide Range Achievement Test (WRAT)], as well as specific instruments for evaluation of health literacy in the special patient groups [diabetic patients, nephrology patients, health literacy in dentistry (TOFHLiD), special age (the Rapid Estimate of Adult Literacy in Medicine (REALM-teen), the Health Literacy Weasure for Adolescents (HELMA)] [3, 12–20]. None of them is specific for determination of pharmacotherapy literacy in the entire population as well as within parents of pre-school children.

The most of instruments were applied in primary care setting (emergency department, waiting room at primary care) [3, 14, 15–18]. Researchers agree that health literacy is related to the context, and different settings require different assessment tools [3].

Recent review identified 109 different health literacy instruments, 37 were non-English, and 72 were in the English language. It was reported that 47% of instruments were a context/content specific, and there is a growth of context/content specific instruments, as it is recognized that one person may have high level of health literacy tested with general health literacy instrument, but it can exhibit lower level of health literacy in the specific condition and in unfamiliar setting [3, 19–21].

With potential limitations of current health literacy instruments as well as an absence of specific one for pediatric medicines use which has to determine the ability of parents to understand information on pharmacotherapy and medicines, we have intended to develop a pharmacotherapy literacy questionnaire, as a specific instrument for assessment of pharmacotherapy health literacy among the parents of preschool children in Serbia (PTHL–SR) and to evaluate the psychometric properties of this instrument.

Methods

The study was a four-stage methodological one, conducted from November 2015 to October 2016 in Belgrade, Serbia. We decided to make survey in a kindergarten setting as it is the easiest way for an access to the parents, and to make the context specific questionnaire. Kindergarten was chosen as a setting, having in mind that in the context of hospital and pharmacy, the parents are often under the pressure due to medical problems of the child, and have no time to fill-in questionnaire. A person was eligible as a parent if he, or she was related to a child living in the household (at least 18 years of age) as a parent, guardian, or a step-parent, speaking the Serbian language.

Our instrument for the determination of pharmacotherapy literacy of parents was made in order to assess the level of knowledge about the use of medicines for the pediatric population, understanding the information provided on the medicines labels, to determine the numerical skills needed for calculation of dose required for a pediatric therapy as well as access to medicines-related information.

In the first phase, we applied the method of relevant literature research. The literature research involved the examination of PubMed database in order to find publications where the questionnaire was used as an instrument to assess health literacy of parents, or knowledge, and understanding of information about medicines and their use in the pediatric population. Our key words for literature search were: health literacy, parents, caregivers, pediatric patients, questionnaire, medicines, liquid oral medicines.

Based on the literature review, we defined four main domains of pharmacotherapy literacy: health knowledge, understanding of health information (written and spoken), numerical skills and access to information about medicines, as these skills are essential for correct use of medicines for children, as presented in Figure 1.

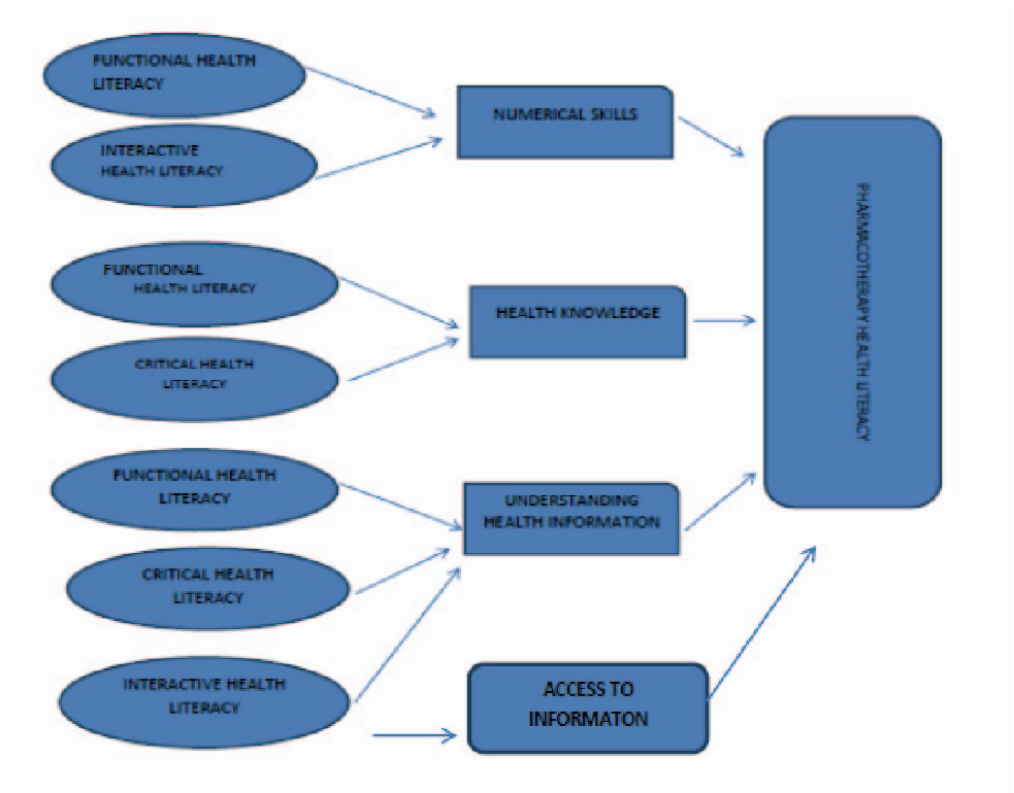

Fig 1. Domains and sub-domains of pharmacotherapy literacy

To determine the qualitative face validity, 10 parents, who were faculty members, were asked to comment about the level of difficulty of items, the obscurity and the proportion of each item.

In the second phase, the content of questionnaire was further defined on the focus group interview [22–24]. The focus group had 8 experts who had to determine the face and content validity of questionnaire. The members of focus group were 3 pharmacists working in the Medicines and Medical Devices Agency of Serbia, 2 professors form Faculty of Pharmacy in Belgrade, one pharmacist working in pharmacy, one doctor-pediatrician and one parent of pre-school child without medical education who is also a professor of Serbian language. Each member of the focus group had to grade each question (with the marks from 1–4), a form of questions and the questionnaire, the clarity of graphical pictures in the questionnaire, suitability of information presented in a question and whether the question should be in the questionnaire. The questions that were given score 1 or 2 as the average score were eliminated. The result of the first interview was elimination of 5 questions and modification of expression of all questions and answers in order to be more clear and more precise. On the second focus group interview, using the same methodology, 3 more questions were eliminated. The content validity ratio (CVR) and index (CVI) were calculated based on the necessity and relevance of each question.

Then, based on the Lawshe's table, the items that scored more than or equal to 0.78 were kept in the scale [25].

The CVI was calculated for each question in the questionnaire, which is the percentage of experts that rated the question as 3 or 4 (based on the rating scale from 1 to 4 where 4 represents excellent fit). It was observed that the CVI value of 1.00 was acceptable for panels of 3, or 4 experts, whereas in the case with 8 experts, the acceptable CVI was fixed at 0.80 or 80% [25].

It was decided that questions meeting the criteria of less than 2 out of 3 methods (66.67% agreement) should be removed. Finally, 16 questions met the criteria of 4 methods fixed for this study, and it was decided to delete 8 questions. After removing the questions, the CVR was calculated.

The CVR is the Content Validity Ratio, and it is zero if a half of experts evaluated the question as essential. Lawshe [25] gave the limit and acceptable values for the CVR related to number of experts (for 8 experts the acceptable CVR is 0.78 and more).

After 2 cycles of focus group interview, our CVR was calculated to be significant [25] and adequate (0.875 > 0.78).

The third phase was pre-testing performed in order to assess the comprehensibility of questions, done in the group of 20 parents who were eligible to fill-in the questionnaire.

In the fourth phase, a quantitative stage, to asses the inter-rater (test – retest) reliability, or consistency among the observational ratings we calculated the Intra-Class correlation (ICC) for the continuous data and Kappa coefficient for the dichotomous data. The Kappa coefficient value was defined by Altman [26]. A split-half reliability testing was also performed to test how many errors in the scores were due to the poor test construction. All questions included the dichotomous data and because of that, they were not subjected to the factor analysis. We considered the Kuder-Richardson (KR20) coefficient scores, which provided an estimate of internal consistency for dichotomous variables (items), which

were interpreted like the Cronbach alpha scores [27]. Ranging from 0.00 to 1.00, KR20 scores must be greater than 0.60 for a measure to be considered reliable [28]. The item difficulty and item discrimination indexes were determined for each question [29]. The item difficulty index is the proportion of subjects (parents) who answered an item correctly. The item discrimination explained the ability of an item to distinguish between the parents who had and the parents who did not have the pharmacotherapy knowledge assessed by the questionnaire. It measured a degree of correspondence between the success in each item and in the whole set of items, and can be computed using a point biserial correlation. The correlation values must be above 0.25 for items to be considered sufficiently discriminating [30]. We calculated the mean, standard deviation (SD), skewness and kurtosis for each question.

The survey was conducted within 10 kindergartens which were located in different municipalities in Belgrade, between March and October 2016. The survey was distributed by an interviewer (SU) who was trained to distribute the questionnaire and basic information about the survey and research. The printed survey was administered to the parents at the scheduled regular parent-teacher meetings in the kindergarten. The parents were allowed unlimited time to complete the questionnaire (on average 10–15 minutes). As an annex to the PHTL-SR questionnaire, the participants had to answer 12 questions related to socio-demographic characteristics. The socio-demographic questionnaire contained 12 questions with information on age, sex, education, number of visits to a pediatrician within one year, information about breast feeding, smoking, presence of chronic diseases at their children and information on self-assessment of health status of the parents.

Before answering the questionnaire, the respondents gave the informed consent to participate in the study. The study was approved by the Committee for Biomedical Research of the Faculty of Pharmacy (321/2, 15.3.2016.). The subjects were excluded from the study if they reported vision problems, and if they decided to stop filling out the questionnaire. All data were collected and analyzed anonymously, in order to keep the privacy of the respondents, as stated in the procedure approved by the Committee for Biomedical Research at Faculty of Pharmacy, Belgrade.

Results

After the examination of publications, a pool of 50 different questions was found. By removing duplicates and similar questions, we found 24 possible questions to be included in the PHTL-SR questionnaire. We translated questions found in the literature and adapted them to fit to the Serbian language regarding the culture aspects. These questions included the items regarding the dosing devices for children (oral syringes, spoons, etc.), use of analgesic and antipyretics, understanding of usual labeling information on packaging and packaging leaflets, where to find relevant information about medicines and knowledge regarding the use of medicines in the pediatric patients. Some questions (5 questions) included graphical picture (photo) of the dosing devices or package.

After 2 cycles with the focus group, 8 of 24 questions were removed, based on calculation of CVR ratio (CVR = 0.875). Thus, our initial questionnaire was approved with 16 questions within 4 groups of questions according to the defined domains of pharmacotherapy literacy: knowledge, understanding of health information, numerical skills and access to information about medicines. Of the 16 questions from the PTHL-RS, 3 questions included graphic of the packaging of medicine for the pediatric use, 2 questions were related to the dosing devices for dispensing syrup for children, one question was entirely taken from the PHLAT questionnaire [8] and translated, and referred to the data on the label of the pediatric OTC medicine.

In the fourth phase, the purposive sample of 300 parents at the kindergartens completed the initial questionnaire to determine its reliability through the internal consistency. In total, 2/3 of parents who were present at the parent meetings at the kindergartens agreed to fill in the questionnaire. The socio-demographic characteristics of the parents were presented at Table 1.

The answers on the questions are dichotomy variables (the correct answers were coded 1 and the wrong ones were coded 0).

The most of parents who participated in the survey were women (80.7%), aged 30–40 years (75.3%), and married (84%) with 2 children (66%). More than two-third (70%) were non-smokers and 61% estimated their health status as very good and excellent (22%). Moreover, a majority of them (40.7%) reported that their first child was breastfed up to 12 months, and 87% of parents reported absence of chronic diseases in their children.

Firstly, all questions from the initial PTHL-RS were analyzed for the KR 20 calculation. The KR20 score for the knowledge was 0.47. After excluding two items (no. 12 and no. 15) because of the poor item fit parameters, the KR20 was 0.54. Since the KR20 coefficient provides the minimum reliability estimates and the difficulty of items (Table 2) was heterogeneous [31], all 14 items were upheld for future analysis. The difficulty of the knowledge items varied from 41% to 97%, averaging 78%. For items 2, 5, 9, 10, 11 and 16 more than 90% of parents answered correctly, indicating that they were relatively easy for participants to answer, whereas less than 50% of respondents answered items 4 and 14 correctly, suggesting that these items were difficult to answer (Table 2). The mean discrimination value was 0.37, ranging from 0.21 to 0.50. Two questions (2 and 16) had a poor discrimination power, but few questions had high discrimination indexes (items 1, 3, 6, 9, 11, and 13) meaning that they were likely to be answered correctly by those who obtained a high score for all questions. The recalculated Split – half coefficient for the questionnaire with 14 questions, was calculated to be 0.542. The mean values, skewness and kurtosis were presented in Table 3. The most items had negative skewness value, meaning that the item values had a tendency to be left skewed.

Table 1

Socio-demographic characteristics of parents

Characteristics	n (%)
Sex	
male	58 (19.3)
female	242 (80.7)
Age (years)	
18–29	17 (5.7)
30–40	226 (75.3)
41–50	46 (15.3)
51–60	11 (3.8)
Number of children	
one child	85 (28.3)
two children	180 (60)
three children	32 (10.7)
four children	3 (1)
Marital state	
unmarried	2 (0.7)
married	252 (84)
divorced	18 (6)
widow	4 (1.3)
common-law	18 (6)
single parent	6 (2)
Education	
no school	0 (0)
primary school	2 (0.7)
high school	81 (27)
higher school (VI grade)	51 (17)
university	159 (53)
PhD grade	7 (2.3)
Employment	
incapable	1 (0.3)
unemployed	35 (11.7)
employed	260 (86.7)
student	3 (1)
pensioner	1 (0.3)
Self-estimation of health status	
very bad	2 (0.7)
bad	2 (0.7)
good	47 (15.7)
very good	183 (61)
excellent	66 (22)
Chronic diseases	
no	261 (87)
yes	39 (13)
diabetes	1 (0.3)
asthma	5 (1.7)
bronchitis	4 (1.3)
cardiac diseases	3 (1.0)
orphan diseases	23 (7.7)
celiac disease	1 (0.3)
allergy	1 (0.3)
other	1 (0.3)
Smoker	
no	210 (70)
yes	90 (30)
Breast feeding of a first child	
≤ 6 months	92 (30.7)
≤ 12 months	139 (46.3)
≤ 24 months	35 (11.7)
≥ 24 months	4 (1.3)
No	30 (10)
Annual visits to pediatrician	
once a year	38 (12.7)
twice a year	43 (15.7)
3 times a year	52 (17.3)
4 times a year	46 (15.3)
5 times a year	48 (16.0)
6 times a year	26 (8.7)
7 times a year	8 (2.7)
8 times a year and more	35 (11.7)

Table 2

Difficulty and discriminatory index

Domain	Difficulty index	Point biserial correlation
Question 1	0.76	0.431
Question 2	0.97	0.219
Question 3	0.85	0.429
Question 4	0.4167	0.363
Question 5	0.9233	0.277
Question 6	0.8	0.495
Question 7	0.6433	0.399
Question 8	0.85	0.319
Question 9	0.9433	0.408
Question 10	0.96	0.379
Question 11	0.91	0.488
Question 13	0.5367	0.402
Question 14	0.42	0.369
Question 16	0.95	0.209

Table 3

Kappa coefficients for answers in PTHL-SR

Domain	Mean ± SD	Skewness	Kurtosis	Kappa coefficient (n = 16)
Question 1	0.76 ± 0.43	-1.22	-0.51	1
Question 2	0.97 ± 0.16	-5.90	33.10	1
Question 3	0.85 ± 0.36	-1.93	1.80	1
Question 4	0.42 ± 0.49	0.33	-1.90	1
Question 5	0.92 ± 0.27	-3.19	8.30	1
Question 6	0.80 ± 0.40	-1.50	0.28	0.444
Question 7	0.64 ± 0.48	-0.60	-1.65	0.815
Question 8	0.85 ± 0.36	-1.97	1.90	0.762
Question 9	0.94 ± 0.23	-3.85	12.90	1
Question 10	0.96 ± 0.20	-4.71	20.40	1
Question 11	0.91 ± 0.29	-2.87	6.30	1
Question 12	0.19 ± 0.39	1.61	0.60	0.762
Question 13	0.54 ± 0.45	-0.14	-2.00	0.444
Question 14	0.42 ± 0.49	0.32	-1.90	1
Question 15	0.86 ± 0.34	-2.12	2.50	1
Question 16	0.95 ± 0.23	-3.99	14.00	1

PTHL-SR – Pharmacotherapy health literacy among parents of pre-school children in Seria; SD – standard deviation.

However, the kurtosis values for 5 questions had the negative value and all other items had the positive values. The inter-rater reliability was demonstrated by the ICC = 0.934 for a variable with a maximum score in the PTHL-RS. As a measure of agreement between the dichotomy variables we used the Kappa coefficient. Eleven answers on the questions showed very good strength of agreement (Kappa = 1), 3 showed good and 2 moderate agreement (Table 2). A split – half coefficient was calculated to be 0.436, which was acceptable. Finally, the questionnaire with the satisfactory psychometric characteristic had 14 questions which are presented at Table 4.

Table 4

Questions included in the final version of constructed questionnaire about pharmacotherapy literacy among parents of pre-school children in Serbia (questions 12 and 15 from the initial version were excluded)

No	Question	Domain	Correct answer (%)
1	What is this medicine (ibuprofen) used for?	Knowledge	76.00
2	What does this medicine contain? (picture of paracetamol syrup)	Knowledge	97.33
3	Would you give aspirin to a child of 6 years if it has a fever?	Knowledge	84.67
4	Your child has otitis and pain. Where do you find information on how much medicine for pain relief to give (per kg or per age)?	Knowledge	41.67
5	What is the highest temperature limit after you give antipyretic to a child?	Knowledge	92.33
6	Pharmacist told you to avoid milk and milk products while taking medicine. What does it mean to you?	Understanding	80.00
7	Avoid sun while taking medicine. What does it mean to you?	Understanding	64.33
8	Keep under 25ºC. After reconstitution, keep refrigerated up to 14 days. How will you store this medicine after reconstitution?	Understanding	85.00
9	You have to give medicine to a child 2 times a day. If your package has 10 items, how many medicines you will have after 3 days?	Numeracy	94.33
10	To mark the dosage for a child of 13 kg on measuring spoon.	Numeracy	96.00
11	To answer how much medicine is inside the oral syringe.	Numeracy	91.00
13	To calculate a dose of oral syrup for child based on dosage regimen per kg.	Numeracy	53.67
14	To interprete paracetamol dosage chart written on package, per weight.	Numeracy	42.00
16	Where did you get an information how much antipyretic to give to your child?	Access	94.67

Discussion

This is the first effort to design a questionnaire for the evaluation of pharmacotherapy literacy among the parents of pre-school children in Serbia. Recent study in Serbia by Jovic-Vranes and Bjegovic-Mikanovic [32] was the evaluation of health literacy screening tool in the primary care patients which involved the population of adults in a medical setting. This survey is a context specific as it was conducted outside the medical setting, in the kindergartens. This study indicates that the PTHL-SR has the validity and reliability necessary for the determination of parents with difficulties in applying and understanding information related to use of medicines. One of the strengths of PTHL-SR is that, beside functional pharmacotherapy literacy, it also addresses the interactive and critical skills. Another important advantage of this study is that it was done outside of the medical setting, in the kindergartens. As health literacy, as well as pharmacotherapy health literacy is a context-specific, applying the questionnaire out of medical settings overcomes the barriers and anxiety the parents may have in the medical setting.

During the construction of questionnaire we included questions that were necessary when using medicines in case of common health problems of children, which together with all mentioned aspects of pharmacotherapy literacy increased the value of PTHL-SR.

Questionnaires for evaluation of health literacy of parents of pre-school children are not completely unknown. The PHLAT (Parental health literacy activities test) [8] is a 20 items questionnaire that covers 3 clinical domains: nutrition-growth/development/ injury-safety, but it is not specific just for pediatric medicines, and it was used in clinical environment. The MedLitRxSE by Sauceda is a general health literacy instrument that assesses skills needed to manage medication properly, not specific for the parents and caregivers of pre-school children because it is also used in clinical setting [20].

The general health literacy instruments (S-TOFHLA, NVS) have limitations as they are not specific and could only serve as the predictive instruments for the assessment of pharmacotherapy literacy of parents. In the study of comparison of NVS and S-TOFHLA [33] it was found that the S-TOFHLA has a ceiling effect as compared to the NVS.

The HELMA (Health Literacy Measure for Adolescents) [3] is a specific questionnaire designed for the special age population from 15–18 years of age, context specific, assessed in non-clinical environment (school). Although age and context specific, it does not include the questions related to the use of medicines. It has 44 items covering 8 different areas.

In comparison to the mentioned specific questionnaires (PHLAT, HELMA, MEDLitRxSE), the factor structure of PHTL-SR is similar to them, as each domain has at least two questions and a reliable construction. It has 4 domains, comparing to the PHLAT which has 3 and the HELMA with 8. In the phase of construction, we used the competences necessary to address the possible cases of use of pediatric medicines and to cover different aspects of medicines use, which increased the validity of PTHL-SR.

As this is the first attempt to construct pharmacotherapy literacy of parents of pre-school children in Serbia, future studies should take into consideration a larger pharmacotherapy group of medicines (not only antipyretics and analgesics) and the parents from rural area as this would lead to the stronger confirmation of psychometric characteristics of PHTL-SR. Having in mind that internet is a frequent source of information for the parents, future studies should also take into consideration the access to reliable information about medicines from the internet.

Limitations

The study was conducted among parents only from the municipalities of the city of Belgrade. The sample was convenient and there was no possibility of data generalization. However, the sample was relatively heterogeneous because parents of different social, cultural, economic and educational environment were included.

Moreover, our questionnaire did not access outcomes of low pharmacotherapy literacy on children's health and this should be addressed in some future studies.

Although the questionnaire had the satisfactory psychometric characteristics, further research has to be performed in the future.

Conclusion

The PHTHL-SR is a reliable instrument for the assessment of pharmacotherapy literacy among the parents of preschool children in Serbia and can be used for the evaluation of different levels of understanding, calculating and accessing different medicines-related information.

Acknowledgements

The work of DK was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No. 41004.

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Received on July 18, 2017.
Revised on December 16, 2017.
Accepted on January 12, 2018.
Online First January, 2018.

ORIGINAL ARTICLE

UDC: 616.8:616-053.2
https://doi.org/10.2298/VSP170417011I

Does neuromotor immaturity represents a risk for acquiring basic academic skills in school-age children?

Da li neuromotorna nezrelost predstavlja rizik za usvajanje bazičnih akademskih veština kod dece školskog uzrasta?

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Abstract

Background/Aim. Studies that investigated the presence of soft neurological signs in terms of neuromotor immaturity and impact on the ability of reading, writing and calculation are rare. At school age, cognitive development in children of the typical population is monitored over, while much less was focused on motor development. This is one of the important reasons for conducting this research. The aim of this study is to determine whether neuromotor immaturity of children poses a risk for the adoption of the basic academic skills in school children. Most authors investigated the presence of dysgraphia, dyslexia and dyscalculia in clinically diagnosed conditions. **Methods.** The data were obtained by testing. To assess neuromotor maturity, The Developmental Screening Test was used. The quality of handwriting was evaluated according to the criteria proposed by Simner and Eidlitz. Quality of reading skills is assessed by the Three-dimensional Reading Test. Evaluation of the presence of difficulties in numeracy was performed by a series of adapted tasks, taken from the Romanian Screening Instrument for Dyscalculia. **Results.** The sample was divided into two groups of pupils according to the results. The groups consisted of students with and without the presence of neuromotor immaturity. There was a significantly higher prevalence of students with difficulties in reading, writing and numeracy in the group of neuromotor immature students. **Conclusion.** The results clearly indicated that neuromotor immaturity of children represents a risk to the adoption of the basic academic skills in children in school age. We believe that the school age is very important time to monitor motor development of a child to the same extent as the cognitive development. In this way, we would have the opportunity to intervene on time and reduce a possibility of school failure. Wider studies in this area are urgently needed.

Key words:
specific learning disorder; child development; child; risk factors.

Apstrakt

Uvod/Cilj. Retka su istraživanja koja istražuju prisustvo blagih neuroloških simptoma u smislu neuromotorne nezrelosti i uticaj na sposobnosti usvajanja čitanja, pisanja i računanja. U školskom uzrastu najviše se prati kognitivni razvoj dece opšte populacije, a mnogo manje motorni, te je ovo jedan od bitnih razloga za sprovođenje ovog istraživanja. Cilj ovog rada je bio da se utvrdi da li neuromotorna nezrelost dece predstavlja rizik za usvajanje bazičnih akademskih veština dece u školskom uzrastu. Uobičajeno se ispituje prisustvo disgrafije, disleksije i diskalkulije kod klinički dijagnostikovanih stanja. **Metode.** Podaci su dobijeni testiranjem. Za procenu neuromotorne zrelosti korišćen je *Developmental Screening Test*. Kvalitet rukopisa je procenjen prema kriterijumima koje su predložili Simner and Eidlitc. Kvalitet čitanja je procenjen Trodimenzionalnim testom čitanja. Prisustvo poteškoća pri računanju procenjeno je adaptiranim Rumunskim skrining testom za procenu prisustva diskalkulije. **Rezultati.** Uzorak je prema dobijenim rezultatima podeljen u dve grupe učenika, onih sa i onih bez prisustva neuromotorne nezrelosti. Nađena je statistički značajno veća prevalencija učenika sa poteškoćama čitanja, pisanja i računanja u grupi neuromotorno nezrelih učenika. **Zaključak.** Dobijeni rezultati nedvosmisleno pokazuju da neuromotorna nezrelost dece predstavlja rizik za usvajanje bazičnih akademskih veština kod dece školskog uzrasta. Zbog toga smatramo da je u školskom uzrastu veoma bitno pratiti i motorni razvoj deteta u istoj meri kao i kognitivni, kako bi se na vreme intervenisalo i kako bi se smanjila mogućnost neuspeha deteta u školi. Potrebna su dalja istraživanja u ovoj oblasti.

Ključne reči:
učenje, poremećaji; deca, razvoj; deca; faktori rizika.

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Introduction

The growth and development of each child follow, to some extent, certain norms [1–5], however, the maturation of certain structures do not happen simultaneously in all children, but has an individual character [6, 7]. This means that in a group of peers, maturation of neural structures in some children comes earlier and in some later. When a child comes to a school setting, i.e., education system, certain expectations appear in front of him. A child should achieve designed educational and behavioral goals. Achieving these objectives will be realized through learning and interaction with peers. The achievements of each child are expressed through the evaluation of acquired materials and behavior. The question is to what extent the immature neurological structure can affect failure in school.

In order to answer this question we need to know in which way we can estimate the level of neuromotor maturity of child. The appearance and disappearance of primitive reflexes in babies show maturation of the central nervous system (CNS). The assessment of presence, or absence of these reflexes determines degree of maturity of the CNS. In this way, one can follow the development of the child and, if necessary, intervene. Primitive reflexes never disappear completely, but should be inhibited, as higher-order centers in the mature brain. They can remain active in damaged higher centers at an early age, such as in the cerebral palsy, or they are present at a later age due to the brain injury and stroke, or as a result of various diseases such as Alzheimer's disease or multiple sclerosis. Goddard [1] states that according to the medical theories, primitive reflexes should not be active in a typical population after six months, and after six months they are considered to be pathological. On the other hand, increasing evidence support the fact that traces of primitive reflexes (residual reflexes) in the general population can remain active even after this age, while any pathological changes that cause this phenomenon cannot be identified [8–13]. Therefore, Goddard [1] defines the presence of primary reflexes in children older than six months as an indicator of neuromotor immaturity. The same author suggests that neuromotor immaturity represents the retention of immature forms of motor control. Goddard [1] states that the retention of immature forms of motor control includes persistent primitive aberrant reflexes in children of school age, as well as posture problems as a reflex of antigravity adaptation of the body and difficulty in balance. The balance represents a dynamic process which is related to relations of different forces of gravity and skeletal muscles. The balance is the final product between the proprioception, vestibular function and vision. The mediator of this process is the cerebellum. Therefore, the author states that neuromotor immaturity means problems in the fields of postural control, coordination, control of movement of the eyeball, perception (vertigo, a sense of direction) and vegetative symptoms (dizziness, disorientation). For the purposes of this study, we accept this kind of approach.

Studies that show that in a number of school-age children remain to persist primitive reflexes to some extent are scarce [8–13]. These studies mainly deal with the population of children with neurodevelopmental disorders and clinically verified behavioral problems, and to a much lesser extent, with learning difficulties and behavioral problems in the typical population of school-age children. Basic academic skills are those skills that are the basis for the success of students in school. According to Wittrock [14] and his extensive review of the relevant literature, there are seven areas of academic skills: writing, reading, mathematics, science, art and aesthetics, moral and educational values and social studies. As Wittrock states, some of these areas, represent the core or basic academic skills for most people. In this paper, as basic academic skills we considered reading, writing and numeracy. The difficulties in acquiring the basic academic skills were seen as “difficulties in reading”, “difficulties in writing” and “difficulties in numeracy”.

McPhillips and Sheehy [12] researched the prevalence of presence of primitive reflexes and motor disorders in the children who had difficulties in reading on a sample of school-age children in Ireland. The analysis showed that there was a difference in the degree of presence of asymmetric tonic neck reflex (ATNR) between the group of children who were the best and the group of children who were the worst readers. This is one of researches that clearly indicates the connection between persistence of primitive reflexes and acquiring reading skills in children. McPhillips, Hepper and Mulhern [10] published the results of research which evaluated the effects of a program that aimed to make the inhibition of ATNR in order to improve reading skills. In a sample of 98 children (27 girls and 71 boys) the program proved to be successful. The degree of presence of reflexes was reduced in the experimental group in which the children received a treatment, and consequently, improved the quality of reading.

Similar results were encountered in the study of Goddard [8]. The methodology proposed by Goddard [8] , unlike previous research, examined not only reflexes but also the presence of many aspects of neuromotor maturity.

The author examined the presence of neurological dysfunctions as a significant factor in the children with dyslexia. A variety of tests investigated the presence of primitive reflexes, postural reactions of ocular-motor functioning, visual-perceptual performance, cerebellar signs, presence of dysdiadochokinesia. In respect of reflexes, there were ATNR and tonic labyrinthine reflex (TLR) in all children. The Moro reflex, symmetric tonic neck (STN), spinal Galant reflex, palmar grasp reflex and hand-to-mouth reflex were present only in a certain percentage. The oculomotor dysfunctions were found in 92% of the sample, in which 83% of children had difficulties in the eye-hand coordination, 42% had difficulties in visual discrimination, 98% had problems in the field of visual-motor integration. McPhillips and Sheehy [12] examined only persistence of reflexes in the children with reading difficulties while Goddard [8] used more comprehensive methodology and examined not only the persistence of reflexes but also other aspects of neuromotor immaturity in the children with reading difficulties. Taylor et al. [13] used the methodology by Goddard [8], but examined some aspects of neuromotor immaturity in the children with the attention deficit hyperactivity disorder (AD/HD).

Most of the research started by isolating a group with specific learning difficulties, and then the presence of neuromotor immaturity aspects were analyzed. This approach is justified when studing clinically diagnosed specific learning difficulties (dyslexia, dysgraphia, dyscalculia) because in this case a characteristic of pathological condition is studied. When a disorder is not diagnosed, we believe that it would be preferable to observe from the opposite perspective, i.e., to examine whether neuromotor immaturity affects the acquiring basic academic skills. We believe that it would be appropriate to first identify the children with neuromotor immaturity, and then deal with the level of maturity and then with the relationship of the level of maturity and the presence of learning difficulties.

The basic academic skills, stage of intellectual development and behavior of children are some of the preconditions for the success in educational and pedagogical outcomes, and thus, of academic achievement. The field of studying neuromotor maturity of children and its impact on these preconditions has not been researched enough. Also an important research related to the relationship of neuromotor maturity and difficulties in numeracy are scarce. Therefore, there is a need to pursue research in this area and to indicate the possible existence of risk of neuromotor immaturity for academic achievements and ability to meet the basic educational and pedagogical goals for the school-age children.

The main objective of this research is to determine the relationship between the presence of neuromotor maturity of school-age children and acquiring basic academic skills.

Methods

The research was conducted in primary schools in the municipalities of Topola and Aranđelovac in Serbia in 2015. A sample consisted of pupils from three primary schools: the primary school "Živko Tomić" (Topola), the primary school "Dušan Radonjić" (Aranđelovac) and the primary school "Ilija Garašanin" (Aranđelovac). The conditions for the inclusion of pupils in the sample were written consents of the institution and parents. First graders were not included in the sample because of the possibility of insufficient data on school achievements, as well as the fact that the teachers did not know them sufficiently to be able to give expert opinions about the school life of pupils. The sample did not include those pupils who are classified in the group of persons with disabilities as well as the pupils who were diagnosed with some of the diseases of the nervous and muscular system, the pupils who were diagnosed with some psychiatric illness, the pupils with intellectual disabilities, autism spectrum of disorders, those with a history of pregnancy, the familial antecedents and the neuropsychomotor development problems.

Before conducting research and sampling, we obtained the written consents of these schools, as well as the approval of teachers, class masters and the parents of children planne ed to be included in the sample. The preliminary sample consisted of 327 pupils of the second, third and fourth grades. Of that number, for 21 pupils, it was not possible to obtain the necessary parental consents. The criteria for inclusion in the sample were not fulfilled for 18 pupils. The final sample consisted of 288 pupils. At the time of testing, 29 pupils were absent from class. For 3 pupils, the teachers did not submit the complete documentation regarding demographic caracteristics. Therefore, the final sample consisted of 256 tested pupils. Of the total sample, 51.20% were males. The pupils were 9.73 year old on average [standard deviation (SD) = 0.88]. The youngest pupil was 7.75 year old, and the oldest was 11.42 year old. The sample was balanced by the number of pupils who participated from each grade, by gender and by age.

The general data on pupils were collected from the general documentation and from their parents. The data on the degree of neuromotor maturity, difficulties in writing, reading and numeracy were collected directly from the pupils through techniques of observation, scaling and testing capabilities.

Assessment tools

The general data were obtained by a specially designed questionnaire. The questionnaire included information on gender, age, school attended, grade, class, dwelling place.

Neuromotor maturity of pupils was estimated by the Developmental Screening Test for Use with Children from 7 Years of Age [1]. The Developmental Screening Test consisted of the Tests of rough muscle coordination and balance [15, 16], the Tests of pathological reflexes [17, 18], the Tests for the assessment of oculomotor function [19] the Tests of visual language recognition [1], the Tests of visual perception and visual-motor integration [20, 21]. Each of the tests scored at five-point scale from 0–4. The total score of all tests was calculated as the sum of scores of all individual tests which was divided by the maximum possible achievement and then multiplied by 100.

The evaluation of the presence of difficulties in writing was done with the use of handwriting samples of children that was obtained by a dictation. The generally accepted pangram was used for the testing in this field [22]. The children wrote the handwriting samples in capital letters in Cyrillic script on white paper without lines. The evaluation of sampled handwriting was performed by the criteria proposed by Simner [2] and Eidlitz [3]. The authors defined 28 types of errors that can occur and be noticeable in a handwriting sample obtained from a child. For each error type, the frequency of occurrence was counted. A total score represented the sum of frequencies of occurrence of all types of errors.

The evaluation of the presence of difficulties in reading was performed by the Three-dimensional reading test [4], which was adapted for the use in domestic conditions at the Institute for Experimental Phonetics and Speech Pathology in Belgrade. The test examined the number of irregularities and errors as well as the understanding of the text. The duration of the test was 5 minutes. The test examined the presence of errors in reading. The total number of errors was recorded on an individual list. When the child read the text, his task was to reword that text. The test had a list of 10 facts which the student should indicate by rewording the text. The individual list recorded the number of words the student said while rewording [4].

The evaluation of the presence of difficulties in numeracy was performed by a series of adapted tasks, taken from the Romanian screening instrument for dyscalculia [5]. The original version of test consisted of 13 tasks. For the purpose of this study we selected 6 tasks found by the factor analysis, that were very important in the context of the test [5]. The tasks were adapted to the educational standards for the subject of mathematics of the Ordinance on education standards for the end of the first cycle of compulsory education for subjects of Serbian language, mathematics and nature and society, but with adjustment to the age levels and grades curriculum. Each task was scored as 0 or 1. The maximum score was 7.

Results

We used the scoring guidelines in order to get the scores on the instruments. The Table 1 shows the results on all used instruments.

Table 1

Scores on all used instruments

Instrument	mean (SD)
DST	17.91 (9.50)
TRMCBR	11.79 (10.63)
TVMI	30.91 (19.46)
TASR	6.71 (8.92)
TVPVMI	21.08 (14.21)
Writing errors	8.58 (9.51)
Reading	
Reading errors	5.03 (5.16)
Remembered facts	3.01 (1.51)
Test of numeracy	4.82 (1.64)

DST – Developmental Screening Test; TRMCBR – Tests of rough motor coordination, balance and reflexes; TVMI – Tests of visual monitoring and integration; TASR – Tests of auditory – speech recognition; TVPVMI – Tests of visual perception and visual-motor integration; SD – standard deviation.

After we obtained the results, we calculated the z-scores for each pupil, for all instruments. The z-scores were calculated relative to the average scores of grades on the individual instruments.

After calculating the z-scores, we created two groups, a group of neuromotor immature pupils and a group of neuromotor mature pupils. If the z-score was equal or less than 1, we considered that the pupil belonged to the group of pupils who had a proper neuromotor maturity. If the z-score was greater than 1, we considered that the pupil had neuromotor immaturity.

To compare the prevalence of difficulties in acquiring basic academic skills and behavior in these two groups, we also calculated the z-scores for each pupil on each instruments. By the same token, the pupils were classified into the group of those with difficulties and those without noted dificulties. As a suitable statistical technique for determining existence of a statistically significant difference in the difficulties prevalence in the group of neuromotor mature and neuromotor immature pupils, we used the nonparametric χ^2 test.

Comparison of groups of pupils with and without neuromotor delay according to the percentage of pupils with difficulties in writing

According to the z-scores in the Test of writing, the pupils were also divided into two groups. The first group consisted of those pupils whose z-scores were equal or less than one. This was a group which we called the group without problems in the field of writing. The second group consisted of those pupils whose scores were greater than one. This was a group with difficulties in writing.

Using the χ^2 test, we compared whether the group of pupils with neuromotor immaturity had a higher percentage of pupils with difficulties in writing than the group of mature pupils. The expected frequency in a cell (25%) was not greater than 5, and we used the Fisher's test besides the χ^2 test. The χ^2 test of independence (with use of the Fisher's test) showed a statistically significant relationship between the status of neuromotor maturity and the status of writing ability, χ^2 (1) = 7.100, p = 0.008. The Fisher's test was significant at the 0.015 level, φ = 0.17. The differences between groups are presented in Table 2.

Table 2

Comparison of groups of pupils with and without neuromotor delay in relation to the level of writing acquisition

Group	WODW	WDW
	n (%)	n (%)
NMM	194 (90.65)	20 (9.36)
NMI	32 (76.19)	10 (23.81)

NMM – neuromotor mature; NMI – neuromotor immature; WODW – without difficulties in writhing; WDW – with difficulties in writing.

Comparison of groups of pupils with and without neuromotor delay according to the percentage of pupils with difficulties in reading (errors)

Using the χ^2 test, we compared whether a higher percentage of pupils with reading difficulties (errors noticeable when reading) was in the group of pupils with neuromotor immaturity than in the group with the neuromotor mature pupils.

According to the z-scores on the Three-dimensional test of reading (reading error), the pupils were also divided into two groups. The first group consisted of those pupils whose z-scores were equal or less than one. This is a group which we called the group without difficulties in reading. The second group consisted of those pupils whose scores were greater than one. This was the group with difficulties in reading.

The expected frequency in a cell (25%) was not greater than 5, and we used the Fisher's test besides the χ^2 test. The χ^2 test of independence (with use of the Fisher's test) showed a statistically significant relationship between the status of neuromotor maturity and the status of reading ability, χ^2 (1) = 29.745, $p < 0.001$. The Fisher's test was significant at $p < 0.001$, φ = 0.34 (Table 3).

Table 3

Comparison of groups of pupils with and without neuromotor delay in relation to the level of reading acquisition (errors)

Group	WODR	WDR
	n (%)	n (%)
NMM	200 (93.46)	14 (6.54)
NMI	27 (64.29)	15 (35.71)

NMM – neuromotor mature; NMI – neuromotor immature; WODR – without difficulties in reading (errors); WDR – with difficulties in reading (errors).

Comparison of groups of pupils with and without neuromotor delay according to the percentage of pupils with difficulties in reading (memorizing facts)

Using the χ^2 test, we compared whether there was a higher percentage of pupils with reading difficulties (memorizing the reading text) in the group of pupils with neuromotor immaturity than in the group with the neuromotor mature pupils.

According to the z-scores of the Three-dimensional test of reading (memorizing facts), the pupils were also divided into two groups. The first group consisted of those pupils whose z-scores were equal or greater than minus one. This was a group which we called the group without difficulties in remembering the text they had read. The second group consisted of those pupils whose scores were lower than minus one. This is the group with difficulties in remembering the text they had read.

The expected frequency in all cells was greater than 5. The χ^2 test of independence showed a statistically significant relationship between the status of neuromotor maturity and the status of capability to remember the text they had read, χ^2 (1) = 12.116, p <0.001, φ = -0.22, p < 0.001 (Table 4).

Table 4

Comparison of groups of pupils with and without neuromotor delay in relation to the level of reading acquisition (memorizing facts)

Group	WODR	WDR
	n (%)	n (%)
NMM	185 (86.45)	29 (13.55)
NMI	27 (64.29)	15 (35.71)

NMM – neuromotor mature; NMI – neuromotor immature; WODR – without difficulties in reading (facts); WDR – with difficulties in reading (facts).

Comparison of groups of pupils with and without neuromotor delay according to the percentage of pupils with difficulties in numeracy

According to the z-scores of the Screening test for evaluation of presence of dyscalculia, the students were also divided into two groups. The first group consisted of those pupils whose z-scores were equal or greater than minus one. This is a group which we called the group without difficulties in the field of numeracy.

The second group consisted of those pupils whose scores were lower than minus one. This is a group with difficulties in the field of numeracy.

The expected frequency in all cells was greater than 5. The χ^2 test of independence showed a statistically significant relationship between the status of neuromotor maturity and the level of ability in numeracy, χ^2 (1) = 46.815, p <0.001. φ = -0.43, p < 0.001 (Table 5).

Table 5

Comparison of group of pupils with and without neuromotor delay in relation to the level of numeracy acquisition

Group	WODN	WDN
	n (%)	n (%)
NMM	198 (92.52)	16 (7.48)
NMI	22 (52.38)	20 (47.62)

NMM – neuromotor mature; NMI – neuromotor immature; WODN – without difficulties in numeracy; WDN – with difficulties in numeracy.

Discussion

Neuromotor maturity and writing ability

The study showed a difference in the prevalence of writing difficulties in the neuromotor mature pupils compared to the group with neuromotor delay. In the group of neuromotor mature pupils, even 90.65% had no difficulties in writing, while this percentage was significantly lower for the group of pupils with neuromotor delays and was 76.19%. These percentages were consistent with researches of foreign authors stating the prevalence of dysgraphia in the typical population of children and amounts between 5%–33% [22]. In our research, we did not clinically determine the presence of dysgraphia but we evaluated the presence of difficulties in writing skills acquisition. In the group of neuromotor mature pupils, the percentige of difficulties in writing was closer to the lower limit of dysgraphia prevalence, while the prevalence of difficulties in writing in the neuromotor immature children was closer to the upper limit of reported prevalence of dysgraphia.

Writing is an ability that belongs to a group of basic academic skills. Pupils are expected to learn writing in first grade. Already in the second grade, the writing acquisition was a default for performing schoolwork and evaluation. Writing serves to express the acquired knowledge planned by the curriculum. Delays in the adoption, or non-adoption of writing can cause a failure in school.

There are no studies dealing with the relationship of neuromotor immaturity and writing. For this reason, we compare obtained results with the results of other research covering the relationships of some of the aspects of neuromotor maturity and writing. Since neuromotor immaturity by definition we gave in the introduction involves persistence of pathological reflexes, problems of postural control, coordination, control of the eyeball movement, perception and vegetative symptoms (dizziness, disorientation), we will observe difficulties in writing in the context of these problems.

Our research clearly shows that there is a greater risk of reporting difficulties in writing with the neuromotor immature children compared to neuromotor mature ones. Our goal was just to point out the existence or absence of risk of neuromotor immaturity.

Other authors have dealt with the relationship of certain aspects of immaturity and writing, however, it should be noted that this issue has been mainly dealt in the population of children with diagnosed disabilities and verified diagnoses but not in the typical population of children.

Marović et al. [23] investigated the relationship of the presence of neurodevelopmental delays in children of typical population of children aged between two and five years and drawing. Drawing contains certain elements necessary for the adoption of writing, but certainly we cannot put a sign of equality between drawing and writing. However, this study is one of the few that is related to the typical population of children and has a similar methodological approach as our research. The authors confirmed that the presence of neurodevelopmental delay negatively affected drawing skill and especially at the age of three to four years, i.e., the period in which the child should begin to draw the first simple geometric figures. Children who have more experience in drawing, especially drawing geometric shapes learn to write much easier [24]. In the neuromotor maturity testing, we used the Visual-motor integration test (VMI), which involved copying geometric shapes and it was a recognized indicator of ability of learning to write. Besides visual-motor coordination, the motor planning, cognitive and perceptual abilities and precise and accurate processing of tactile and kinesthetic information are indicators for the adoption of writing [25]. Most of these aspects is contained in the concept of neuromotor immaturity in the way we defined it.

The presence of residual reflexes may also negatively affect learning how to write. Abnormal and primitive postural reactions negatively impact on the eye-hand coordination that is much needed for the learning to write. Literature recognizes residual ATNR as the cause for not adopting writing skills. When conducting research, we saw an example of a 12-year-old boy with the presence of residual ATNR. When writing, the boy was sitting on the left leg and his arm was slightly flexed. His head was turned slightly to the right and he read mainly by left eye. A pencil grip was too tight. The boy was so focused to maintain control over his own body, that he was not able to listen, visually monitor, nor to take notes. Residual ATNR, according to the literature, may compromise the hand-hand control when head is turned to one side, the ability of crossing the midline of the body which affects the left-right orientation [26, 27]. It can also impair control of the hand in writing [28], and visual skills necessary for reading and visual tracking [29, 30]. If residual symmetrical tonic neck reflex (STNR) is not repressed in school age, this can negatively affect posture when sitting, and to a great extent determines the quality of writing. If the residual STNR is present, the child does not have the ability to sit still and can lead to coordination problems. Residual palmar reflex can jeopardize the correctness of grip in writing.

The influence of eye movement and vestibular ocular reflex on reading and writing is verified [31]. Sales and Colafêmina found that the pathological changes of saccades and pendulous movements of the eye that serve tracking opened up the possibility for appearing the difficulties in reading and writing. The survey was conducted on a sample of children aged from 9 to 14 years with and without disabilities in reading and writing.

One study, that examines the verified pathological conditions in children, compared three groups of children, clumsy, children with dysgraphia and children of typical population according to the quality of handwriting and perceptual-motor skills [32]. Of all tested perceptual-motor skills only the visual-motor integration predicted the quality of handwriting in the whole sample of 59 ten-year-old children. It was confirmed that the quality of handwriting is very strongly associated with the visual integration, visual perception and tracking at the level of the entire sample. What is interesting and may help to explain the results of our research is the fact that children with dysgraphia had the lowest scores on the tests of visual-motor integration compared to the other two groups of children.

Persistence of reflexes, problems with coordination, posture, balance, visual perception and integration can affect the acquiring and quality of writing in children. All these difficulties, when are not an integral part of a verified pathology in the children, can be subsumed under a single umbrella term – neuromotor immaturity. Based on these results and the results of other authors supporting and valorizing the results of this study, we can conclude that each of the aspects of neuromotor maturity individually can have an impact on the acquisition of writing, and neuromotor immaturity of children undoubtedly represents a risk to the acquisition of writing in the school- age children.

Neuromotor maturity and reading skills

In the research, we evaluated two aspects of reading, namely, errors in the production from the reading text and memorizing facts from the text they had read. Analysing errors of pupils made in reading we found a difference in the quality of reading in the neuromotor mature and neuromotor immature children. The vast majority (93.46%) had a very small number of errors in reading, so they practically have no difficulties in reading. In the neuromotor immature children, only 64.29% of them had no difficulties in reading. The difference in the prevalence of reading difficulties is very large and statistically significant.

Analyzing the second aspect of reading that we examined, i.e., memorizing facts from the reading text, we found the similar tendencies in the prevalence of difficulties between the two mentioned groups of children. In the group of neuromotor mature pupils, the vast majority, or 86.45% of them had no difficulties in memorizing the text they read. Only 64.29% of the pupils belonging to the group of neuromotor immature pupils had no difficulties to remember facts from the reading text.

Neuromotor immaturity of children is a risk for learning how to read in children. Possible reasons can be sought in the relationship of certain aspects of neuromotor maturity and reading skills in children of school age.

Poorer performance on a variety of "cerebellar" motor tasks were reported in dyslexia [33]. Cerebellar dysfunction is an underlying cause of developmental dyslexia, which is diagnosed when there is an unexpected discrepancy between a child's reading ability and their cognitive skills. Cerebellar dysfunction leading to a procedural learning deficit could be an explanation of deficiency in acquiring literacy skills [33]. Also, the magnetic resonance imaging (MRI) studies of cerebellar differences in dyslexic children confirmed reduced grey matter in right lobule VI and reduced grey matter in left VI. It was found that the fluency component of dyslexia is associated with abnormal activation in the right lobule VI [33].

One of longitudinal studies examined how the development of speech and motor skills at the age of about the second year of life determined the ability of learning how to read [34]. The author found that the development of motor and speech skills determine reading ability at school age, but it was insufficiently at this age to make a diagnosis, classification, or to indicate a potential risk for not adopting reading. Two facts that arise from this study confirmed the results of our research. Firstly, the motor and speech skills determine reading skills. The neuromotor maturity assessment includes the evaluation of motor skills and visual-linguistic recognition. Secondly, between the second year and school age, there are more years during which the child is actively developing. However, we believe that, when it comes to the typical population, influences of motor and speech skills should not be considered separately, but as a whole, along with other aspects of neuromotor maturity.

Difficulties in reading are also a broad term and may include difficulties of verbal expression, recognition and comprehension of symbols and remembering reading text.

Researches of residual reflexes support our results. When there is a residual ATNR, it prevents proper tracking, and eye tracking forward and backward (saccades), which is absolutely necessary to read. Crossing the midline, can be impaired, and the child tracks line by whole his head instead by view. The presence of ATNR in school age may prevent eyes coordination function which induces blurred vision and diplopia. Also, its presence means that adequate connection between the brain hemispheres (*corpus callosum*) do not develop and the dominance of the brain functions do not develop properly. These links mature naturally at the age of 6.5 to 7.5 years and there are often mirrored letters to that period, but not after being 8. Inadequate development of *corpus collosum* restrains preference of skills for which the right side of the brain should be responsible the left side contributing to the difficulties of children with dyslexia. There are often present difficulties in the remembering, spelling, learning, sense of direction. Without the developing the dominance of hemispheres, the lateralization and development of a sense of direction are prevented. Centers for decoding language are in the left hemisphere of the brain, thus ATNR can inhibit the development of language skills such as reading and writing. ATNR is stimulated by the vestibular system, which means that this system would be less developed, resulting in problems of balance and coordination. Many previous studies confirm that the use of cerebral-vestibular tests in combination can, to some extent, detect learning disabilities such as dyslexia and attention deficit disorder (ADD) [35, 36]. Levinson [36] even concluded that dyslexia and ADD are a reflection of deeper cerebral-vestibular determinants. When tonic labyrinthine reflex (TLR) is restrained, it often affects the problem of the spatial orientation and especially confusion on distance of things, what is in front and what is behind. In reading, for example skipping lines may occur.

There are studies that explored the potential impacts of postural control on reading skills. Loras et al. [37] state that there are opposing views on whether postural control affect the quality of reading. The studies that confirm this influence, Loras et al. [37] state, do not deal with the nature of this relationship nor explain it. Loras et al. [37] find in his research that there is no statistically confirmed link between the abilities of balance and reading results on the cognitive tests or tests of attention. It should be noted that the sample consisted of 100 people aged above 20 years.

This study affirms that not all aspects of neuromotor maturity affect the reading skills in children, but the vast majority do. Our results confirm that neuromotor immaturity of children poses a risk to the acquisition of reading in children. The review of studies that deal with the relationship of aspects of neuromotor immaturity and reading, largely affirm the results.

Neuromotor maturity and numeracy skills

The research showed that a number of pupils have difficulty in numeracy. In the group of pupils who were neuromotor mature, 92.52% of them had no difficulties in numeracy, while in the group of pupils with neuromotor immaturity only 52.38% of them had no difficulties in numeracy. Since we used tasks from the test designed for screening dyscalculia to detect difficulties in numeracy, we would compare the obtained result with the results of other studies in the field of developmental dyscalculia. The prevalence of developmental dyscalculia is 3%–6% [38]. In our study, it was found that there were problems of numeracy in 7.48% of children in the group of neuromotor mature children. Possible reasons for a slightly higher percentage than that reported in the literature can be found in the fact that the cause of dyscalculia can often be explained by the presence of dyslexia. Also, the difficulties in numeracy were much more common in the children from rural areas as compared to those from urban areas. Our sample consisted of children mostly from rural areas.

In the children with neuromotor immaturity, the difficulties in numeracy were present in a large percentage, and therefore, the neuromotor immaturity represents a risk for the acquisition of numeracy skills. The question is whether the risk is direct or indirect. The acquisition of mathematical operations and numbers is very complex. Mathematical operations are diverse and involve a whole range of skills that are required for their performance. It is believed that the per-

formance of arithmetic operations requires primarily cognitive and much less motor skills. In this sense, we can consider that reduced cognitive abilities can be the most important direct causes for difficulties in performing arithmetic operations. We would try to eleborate the results we got and establish a potential connection between the neuromotor immaturity and presence of difficulties in numeracy.

In 1970, Ladislav Kosc [39] defined developmental dyscalculia as a structural disorder of mathematical skills which origin stemmed from genetic or congenital disorders of those parts of the brain that were direct anatomical-physiological substrates of maturation of mathematical abilities in accordance with age, without concurrent disorders of general mental function [39]. Kosc actually suggested that causes of difficulties in numeracy could be twofold, whether as a result of underdeveloped mental functions, or as a result of structural brain disorders.

It is known that a dysfunction in any hemisphere of the brain can lead to a reduced ability of acquisition of numeracy skills. The difficulties are more pronounced if the left hemisphere is compromised. It was found that the left hemisphere is in conjunction with constructive dyspraxia, poor auditory and visual discrimination and motor coordination. Within neuromotor maturity, using the Tansley standard visual figures test and the Bender test, we examined, among other things, a visual discrimination. The Tandem walk and Fog walk test require a motor coordination during the execution of the tasks. Within the assessment of neuromotor immaturity, we examined the ability of auditory discrimination. In case of our study, we do not assume structural changes in the form of brain damage, but we talk about immaturity of structures to take over functions completely and partially.

Dysfunctions of the right hemisphere in the group of children who do not show signs of structural abnormalities the MRI and computed tomography (CT) were manifested as defects in the domains of graphomotor skills, slow cognitive and motor performance, although the reading skills are preserved. Dysfunctions of right hemisphere involve various difficulties including difficulties in spatial perception. Many of the symptoms that are associated with dysfunction of the right hemisphere may also occur as a result of dysfunction of the vestibular system and associated pathways that support the processes of visual perception in the right hemisphere [9]. Risey and Briner [40] examined the relationship of the presence of central vertigo and dyscalculia and found that patients with vertigo had difficulties to count backwards and even when they were aware of making a mistake, they continued to repeat the process. Hence, the difficulties of balance may affect the acquisition of certain operations with numbers. The patients in this research also had difficulties with the mental arithmetic and central auditory processing. As part of our research, we evaluated the balance, and within the assessment of acquired mathematical operations there were tasks to repeat numbers backwards. Therefore, there is a possibility that the immaturity of vestibular function leads to a dysfunction in the field of visual and auditory processing and maybe to the difficulties in the process of mental sequencing.

Regarding the relation of persistence of residual reflexes and reporting difficulties in numeracy, we did not find in the literature that any author indicates a direct connection of these phenomena. Some of the known facts could be a link between these two phenomena. The knowledge of own body parts, especially hands and fingers is very important for learning the concept of number. The child firstly meets his/her body. Acquisition of numbers to 5 is closely related to counting the fingers of the hand. Later, the counting of physical objects comes. The speed of development of hand and grasp may have an indirect connection with the acquisition of numbers. When assessing the reflexes, the balance has a major impact on the performance of tasks and evaluation whether the residual reflex is present, or not. We have already mentioned the example of research that linked the central vertigo and dyscalculia.

Neuromotor maturity and acquisition of arithmetic operations are very complex. The obtained results suggest that there is a risk that the neuromotor immaturity bears for the acquisition of basic academic skills and acquisition of mathematical operations. We listed some of researches that support the results and indicate the nature of the risk. Such researches are scarce and very much desirable in the future.

Conclusion

There are studies referring to the verified neurodevelopmental delay and special learning disabilities such as dyslexia, dysgraphia and dyscalculia. On the other hand, research that relates to the impact of soft neurological signs and neuromotor immaturity on the possibility of the acquisition of reading, writing and numeracy are very rare. Our research gives a complete picture of the risks of neuromotor immaturity for the acquisition of these skills. This study clearly indicates that the risk of difficulties in reading, writing and numeracy is significantly higher in neuromotor immature children compared to those school children without delays. We hope that this research will support the emergence of new forms of treatments and rehabilitation procedures within the system of education. The results indicate that the neurological and motor characteristics must be considered on an equal footing with cognitive capacities in school settings because they determine the quality of basic academic skills of the child that are necessary and represent a prerequisite for learning process and achieving educational goals.

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Received on April 17, 2017.
Revised on October 13, 2017.
Accepted on January 15, 2018.
Online First January, 2018.

PRACTICAL ADVICE FOR PHYSICIANS

UDC: 615.03:616.8]:615.213
https://doi.org/10.2298/VSP170910180J

Where next for antiepileptic therapeutic drug monitoring?

Kuda dalje sa terapijskim monitoringom antiepileptika?

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Key words:
anticonvulsants; epilepsy; drug monitoring; dosage forms; plasma; saliva.

Ključne reči:
antikonvulzivi; epilepsija; lekovi, monitoring; doziranje, oblici; plazma; pljuvačka.

Introduction

Therapeutic drug monitoring (TDM) is a clinical procedure of adjusting drug dose according to its measured concentrations in plasma or other biological fluid. It makes sense if the following conditions are met: there is a correlation between the plasma concentration and drug effect, concentrations in biological fluids vary significantly between the subjects (high inter-individual variability), the drug has a narrow therapeutic window, a suitable method of measuring drug concentrations exists, and the drug effect cannot be easily and precisely measured in clinical practice [1, 2]. However, in order to make an adjustment of the drug dose according to its plasma concentration, the therapeutic range of plasma concentrations should be known, based on the results of previous clinical trials. Some of the first and second generations of anticonvulsants fulfilled all these conditions, and the TDM of these drugs is currently conducted in clinical practice as a routine procedure: phenobarbital, phenytoin, carbamazepine and valproic acid. Classification of anticonvulsant drugs according to the generations (historical development) is usually made in the following way: the first generation drugs (phenobarbital, phenytoin, carbamazepine, ethosuximide and valproic acid), the second generation drugs (lamotrigine, topiramate, oxcarbazepine, tiagabine, pregabaline, vigabatrine, zonisamide, gabapentin, felbamate and levetiracetam) [3] and the third generation anticonvulsants (eslicarbazepine, lacosamide, perampanel, brivaracetam, rufinamide, retigabine and newer drugs) [4].

The true clinical impact of TDM in the patients with epilepsy was rarely investigated in clinical trials [5]. One of rare clinical trials published up to date [6] investigated the impact of TDM on the patients with newly diagnosed epilepsy (partial or idiopathic generalized nonabsence epilepsy) for carbamazepine, valproate, phenytoin and phenobarbital. TDM kept plasma concentrations within the therapeutic range in more patients than in the control group, but neither 12-month remission rate, fraction remaining seizure free since an initiation of treatment nor the time to the first seizure, or 12-month remission were significantly influenced by TDM. The adverse effects rate was also very similar in the TDM and the control group. The authors concluded that the TDM should be reserved for selected patients and special situations.

For many of the second and third generation anticonvulsants, therapeutic plasma range it is still either unknown or uncertain [7–10]. In such cirsumstances measuring the plasma concentration of a drug is only of an exploratory character, i.e., the precise recommendations about the amount of dose increase or decrease could not be given. However, the clinicians still can adjust the dose of an anticonvulsant in some particular patient, using both information about the measured plasma concentration and clinical presentation of patient.

Even for anticonvulsants with the well- established TDM, only the total drug concentration in plasma is measured routinely, which may mislead a prescriber. If hypoalbuminemia is present, the total drug plasma concentration could be within the therapeutic range, but free drug may be highly elevated and cause serious toxicity, as reported for valproate [11]. Measuring plasma concentrations of free fraction of anticonvulsants should become a standard, and the therapeutic ranges of free drug should be established if we want to increase the precision and usefulness of TDM.

These, and several other issues remain unresolved with the TDM of anticonvulsants. The aim of this review was to describe them in sufficient detail, offering basis for planning the further developments of TDM.

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Issues with therapeutic drug monitoring of phenobarbital, phenytoin, carbamazepine and valproic acid

Early after introduction of TDM as a routine procedure in the patients prescribed with first generation anticonvulsants, the clinicans became aware of several issues that make difficulties when deciding about a dose adjustment after the plasma concentration of the drug is obtained [12]. First, they understood that the therapeutic ranges of plasma concentrations could not be applied to all patients, without taking into account a seizure type and frequency, yet the specific data were lacking to guide the prescriber. An importance of measuring an unbound fraction of drug within plasma was also perceived, as well as an influence of numerous factors which may change pharmacokinetics of anticonvulsants and make dose adjustments wrong if not considered in an individual patient (genetic polymorphisms, drug-drug and drug-food interactions, co-morbidities, noncompliance, or a special physiological states like pregnancy). It became clear that in order to choose the right dose regimen at least in some patients (sometimes wrongly classified as „therapy resistant"), it is necessary to make an objective estimate of long-term seizure frequency, adverse effects on motor and intelectual functions, quality of life, perhaps continuous electroencephalography (EEG) monitoring and measurement both of total and unbound anticonvulsant plasma concentrations. This led to an idea that an individual therapeutic threshold value (ITTV) should be searched for in a patient, or the minimum steady-state anticonvulsant concentration that, in that patient, results with the complete control of seizures and a lack of significant adverse drug reactions [13]. A clinician should prescribe the lowest dose of anticonvulsant that achieves through plasma concentrations above the ITTV; however, establishing the exact ITTV in a patient is difficult task to achieve in clinical practice, and a great majority of clinicians still relies on the population therapeutic range of plasma concentrations instead. Nevertheless, we should start a treatment of patient having the population therapeutic range in mind, but then, if possible, to adjust an interpretation of measured plasma concentrations to the individual characteristics of patient and the influences he/she was exposed to, as mentioned before.

Several studies showed that was not appropriate to consider only one range of steady-state plasma concentrations as therapeutic for certain anticonvulsant, since it depends on the type and the severity of epilepsy, measured by the seizure frequency before the treatment [14, 15]. In theory, if specific therapeutic ranges of cetrain anticonvulsant are established for each type of epilepsy and severity class, a prescriber would have a clear target steady-state concentrations to achieve in every patient, so the use of information about the measured plasma concentration for adjusting the dose regimen would be straightforward. Unfortunately, up to date, the well-designed and large enough studies have aimed to establish the type- and severity-dependent steady-state plasma therapeutic concentration ranges were not conducted, and we are left without this knowledge.

Phenytoin, carbamazepine and valproic acid were the first anticonvulsants for which an advantage of measuring unbound plasma concentration over the total plasma concentration was shown in clinical practice, especially in such situations when a patient had the low albumin levels [16]. However, a direct measurement of unbound drug is technically more difficult and demanding, and some clinicians revert to the calculation of adjusted phenytoin plasma concentration based on the measured total plasma concentration, albumin level and Sheiner-Tozer equation. Although relying on the fact that the adjusted plasma concentrations give better results than the total plasma concentrations when deciding about phenytoin dose, using the measured free (unbound) plasma concentration of phenytoin for dose adjustment remains the gold standard for TDM of this drug [17]; unfotrunately, we are far from achieving that standard in a majority of TDM units, even in the developed countries.

Therapeutic drug monitoring

TDM is only possibly useful for the second-generation anticonvulsant lamotrigine, since a correlation between plasma concentrations and effect was not proven unequivocally, and the therapeutic range was loosely defined [18, 19]. There is similar experience with topiramate, another second-generation drug, as its average plasma concentrations were not significantly different between the responders, non-responders and patients with toxic reactions [20, 21]. An unproven plasma concentration-effect correlation and wide, or the unreliable therapeutic range are common place for all other second-generation anticonvulsants, including tiagabine [22], pregabaline [23], vigabatrine [24], zonisamide [25], gabapentin [26], felbamate [27] and levetiracetam [25]. Vigabatrine is not significantly bound for plasma proteins and acts as an irreversible enzyme inhibitor of gamma-aminobutyric acid aminotransferase, so TDM of this drug is considered useless [24]; the additional obstacles for TDM use in the patients on levetiracetam therapy are very wide therapeutic range and minimum side effects [25]. Measuring free (protein unbound) plasma concentrations of second-generation anticonvulsants within the framework of TDM was not even attempted.

For a majority of the third generation anticonvulsants data about the clinical usefulness of TDM are missing [28], or only point to a large inter-individual variability, which is just one of the conditions that should be met (e.g., eslicarbazepine, lacosamide) [29, 30]. A reliable therapeutic range of plasma concentrations is not established for any of these drugs, although the assays for the measurement of plasma concentrations are developed and validated for many of the newest anticonvulsants, like perampanel [31], or rufinamide [32].

It is clear that the use of TDM for the first-generation anticonvulsants could be improved and enlarged, and also many of second and third-generation anticonvulsants have true potential to become drugs where routine use of TDM is mandatory. However, there are several obstacles to achieve this ideal situation, and both the technological developments and substantial workforce along with the financial investments are necessary to overcome the obstacles. Without at-

tempting to be systematic, the obstacles and ways to remove them could be listed as following: unknown seizure type and severity-specific therapeutic ranges of plasma steady-state concentrations of almost all anticonvulsants; a lack of simple, rapid and inexpensive methods for measuring plasma concentrations of free drug (unbound for plasma proteins); lack of noninvasive methods for measuring steady-state concentrations of anticonvulsants which correlate well with therapeutic and toxic effects; a lack of evidence that TDM for each particular anticonvulsant is improving the relevant outcomes of treatment, as the seizure control and quality of life, as well as the decreasing adverse events rate and overall treatments costs.

Seizure type and severity-specific therapeutic ranges

The only reliable way to establish a seizure type and the severity-specific therapeutic range of steady-state plasma concentrations of an anticonvulsant is to conduct a properly designed and adequately powered clinical trial which would include the patients with various seizure types and severity of epilepsy. Some of the researchers in their clinical trials analyzed the effects of anticonvulsants in the various subgroups according to the seizure type and noted different effects with the same plasma concentrations [33], but the attempts were rarely made to establish therapeutic ranges, usually because the subgroup analyses were underpowered, but also because the statistically significant concentration-dose relationshipis sometimes nonlinear [34]. However, an indirect proof that there must be the seizure type – the specific therapeutic ranges are dosing recommendations of topiramate and many other anticonvulsants in their Summaries of product characteristics, which are dependent on a seizure type [35]. Recently, a comprehensive systematic review was made (810 full-text articles reviewed, and data extracted from 163) with an aim to establish a therapeutic index for anticonvulsants based on the published clinical data for five anticonvulsants, but it was possible only for phenytoin, phenobarbital and valproate, regardless of the seizure type or severity [36]. This situation could be improved, at least partially and for new anticonvulsants, if the guidelines for clinical trials include recommendation that one of the study outcomes should be establishing the seizure type (and if possible severity and the specific therapeutic range (or index) of plasma concentrations. Another way is more sophisticated use of observational data for antiepileptics where TDM has already been already done in clinical practice, through pooling the data from different studies and use of statistical techniques to eliminate a bias.

Measuring plasma concentrations of free drug

Measuring concentrations of free (unbound drug) *in vitro* is usnally performed by one of the following methods: equilibrium dialysis, ultrafiltration and the Hummel and Dreyer method for the gel permeation chromatography. However, when measuring the free drug in plasma, only the equilibrium dialysis and ultrafiltration are used, because the gel permeation chromatography has high measurement „noise" due to the abundance of the small-size molecules in plasma [37]. Perhaps the most suitable for use in clinical practice is the ultrafiltration method. Although up to date, enough simple, rapid and inexpensive method for measuring the free plasma concentration of anticonvulsants was not developed for the routine TDM, it should not be too far away, since something similar was already developed for measuring the free plasma concentrations of ten beta lactam antibiotics in the critically ill patients [38].

Noninvasive methods for measuring steady-state concentrations of anticonvulsants

Measuring the concentrations of anticonvulsants in saliva was long ago seen as potentially a very suitable method for TDM, as it is noninvasive and easy to repeat as many times as necessary from the point of view of patients, who certainly would be much more compliant with a such method than with TDM based on the blood samples (injection phobia is highly prevalent in every social milieu) [39]. Besides, the physicans always prefere painless methods, if available [40]. It was early proven for the first-generation anticonvulsants that the intra-individual variability of measured concentrations in saliva was small enough to be acceptable for TDM purposes [41], and later significant correlation was found between total plasma concentrations and concentrations in saliva [41] when anticonvulsants were used in a monotherapy, or a combination, but not after the polytherapy. In general, concentrations of anticonvulsants in saliva make 10%–40% of plasma concentrations, what is enough for routine TDM [40]. It was recently shown that the valproate concentrations in saliva correlate well with concentrations of free valproate in plasma [42], which is promising because the free drug in plasma is considered to be active, and as mentioned earlier, in good correlation with the therapeutic effect. However, more recent studies showed that the salivary valproate concentration was not reliable to be used for TDM, while the following anticonvulsants could be measured in saliva and steady-state values used for TDM: carbamazepine, ethosuximide, clobazam, gabapentin, lamotrigine, lacosamide, levetiracetam, oxcarbazepine, phenytoin, phenobarbital, primidone, topiramate, and zonisamide [43]. Although possible, TDM based on salivary concentrations is far from the routine use – much remain to be done on establishing a therapeutic range in saliva for the abovementioned anticonvulsants, including specially designed clinical studies (both clinical trials and observational studies) for each drug separately.

Therapeutic drug monitoring and treatment outcome

Ultimate goal of TDM is to improve treatment outcomes of anticonvulsants and minimize occurrence of adverse effects. However, the achievement of that goal was surprisingly rarely proven in clinical studies even with the first-generation anticonvulsants. It was demonstrated for some anticonvulsants that TDM resulted in the decreased seizure frequency [44], or that the complete seizure control was

achieved in some patients previously considered a therapy resistant [45]. TDM is also very useful to discover the non-compliant patients [46] and a relationship with the decreased frequency of adverse effects, which was shown in a few studies [47]. A decrease in the overall treatment costs with the use of TDM was not shown, but several studies pointed to a generation of unnecessary costs if TDM was improperly used (taking blood samples out of the steady-state, misinterpretation of results, etc.) [48]. The effects of TDM on quality of life of patients with epilepsy were not investigated, either. There were some studies, too, which failed to find any connection between TDM and treatment outcomes, or adverse effects [49]. Considering the scarcity and incompleteness of published data, there is an obvious need for new clinical trials, or observational studies which would explore a relation between the TDM of anticonvulsants and the treatment outcomes (including costs and quality of life) / adverse effects, both for the old and new drugs that fulfill the criteria for TDM.

Availability of point of care of therapeutic drug monitoring tools

Although the intense development of point of care (POC) tests were introduced in the past for TDM of anticonvulsants, their use did not widespread because there was not enough patients to justify the costs at that time and the quality assurance was questionable [50]. However, rapid technological advances in nanosciences and biosensors created an opportunity for the development of reliable and less expensive point-of-care tests for measuring concentration of anticonvulsants in the blood, or saliva and obtaining immediate results [51, 52]. This advancement would be useful especially in the evaluation of possible toxicity of an anticonvulsant, as it is when we need the information about drug concentration as soon as possible. Another possible prospect of POC tests is that the establishment of individual therapeutic threshold value could become feasible for more patients than it was case until now (mesurements could be done at the patient's home, during weekends and holidays, and when the staff trained for venepunction is not available). With POCs, we could capture the concentrations of anticonvulsants immediately after, or before a seizure event, and also when a patient is changing diet, or has the drugs unrelated to epilepsy prescribed. All these advantages should help us to tailor a better anticonvulsant therapy for an individual patient, and hopefully, improve therapeutic outcomes.

Conclusion

Despite a relatively long history of TDM use within the framework of epilepsy treatment, we are using only small part of possibilities it offers, in the first place because of lack of specific knowledge. In order to use full capacity of TDM in the future for maximum benefit of patients with epilepsy, we need to establish the seizure type and severity-specific therapeutic ranges for those anticonvulsants where TDM has clinical significance, as well as to prove the positive effects of TDM on a wide spectrum of treatment outcomes. The development of non-invasive TDM methods, the point-of-care tests and reliable methods for routine measurement of free drug concentrations in plasma are also the areas where progress could empower TDM of anticonvulsants and bring new qualities. However, we should acknowledge that for many years, the TDM has been successfully used for adjusting doses of the first-generation anticonvulsants, provided that important confounding factors were taken into account, like hypoalbuminemia, hypervolemia, acid-base disequilibrium, and others.

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Received on September 10, 2017.
Revised on November 18, 2017.
Accepted on November 20, 2017.
Online First December, 2017.

CASE REPORTS

UDC: 616.321-006-053.31-089
https://doi.org/10.2298/VSP171016176M

Congenital hairy polyp – A case report

Kongenitalni dlakavi polip

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Abstract

Introduction. Hairy polyp is a rare malformation which consists of mesodermal and ectodermal elements. It is the most common congenital nasopharyngeal tumour. The clinical symptoms depend on the size and localization of the mass. Early surgical excision results in permanent cure. **Case report.** The child was born from regularly controlled pregnancy. Prenatally, in the 24th week of gestation, *epulis gigantocellularis* was suspected, magnetic resonance imaging confirmed existence of protruding mass. Immediately after the birth, a tissue formation connected with the hard palate was observed protruding out of the mouth, as well as split of soft palate and tongue. In the first day of life the child was intubated and a mechanical ventilatory support started. In the fourth day of life, tracheostomy was performed. In the 40th day of life, the tumor mass was entirely excised with the reconstruction of the existing deformity of the mouth. The histopathological analysis confirmed the diagnosis of hairy polyp. In the fifth month of life, a percutaneous endoscopic gastrostomy was placed. At the beginning of the second age, the split of tongue and soft palate was sewn. With the establishment of normal swallowing, decannulation and closing of tracheostomy were performed and then gastrostomy was closed. **Conclusion.** Although the hairy polyp is a rare tumor, it must be included in the differential diagnosis of pharyngeal mass in the neonatal period. If there are no associated anomalies and if complete surgical resection of the tumor is performed, further course of the treatment will be favourable.

Key words:
cleft palate; diagnosis; histological techniques; infant, newborn; nasopharyngeal neoplasms; polyps; otorhinolaryngological surgical procedures.

Apstrakt

Uvod. Dlakavi polip je retka malformacija koja se sastoji od mezodermalnih i ektodermalnih elemenata. To je najčešći kongenitalni tumor nazofarinksa. Klinički simptomi zavise od veličine i lokalizacije polipa. Rana hirurška ekscizija rezultuje potpunim izlečenjem. **Prikaz bolesnika.** Dete je rođeno iz redovno kontrolisane trudnoće. Prenatalno, u 24. gestacijskoj nedelji, postavljena je sumnja na postojanje *epulis gigantocellularis*-a. Urađena je magnetna rezonanca i evidentirana masa koja je protudirala iz usne duplje. Odmah po rođenju je uočena tkivna formacija vezana za tvrdo nepce, koja prominira van usta, kao i rascep mekog nepca i jezika. U prvom danu života dete je intubirano i započeta je mehanička respiratorna potpora. U 4. danu života izvršena je traheostomija, dok je u 40. danu života tumorska masa u celini ekscidirana, uz rekonstrukciju postojećih deformiteta u usnoj duplji. Histopatološka analiza je utvrdila postojanje dlakavog polipa. U petom mesecu života deteta izvedena je perkutana endoskopska gastrostoma. Na početku druge godine života rascep nepca i jezika je hirurški korigovan. Po uspostavljanju adekvatnog gutanja sprovedena je dekanilacija i zatvorena traheostoma i u daljem toku gastrostoma. **Zaključak.** Iako je dlakavi polip redak tumor, mora biti uključen u diferencijalnu dijagnozu faringealnih masa u neonatalnom periodu. Ukoliko nema pridruženih anomalija i ukoliko je izvršena kompletna hirurška resekcija tumora, dalji tok i ishod lečenja je povoljan.

Ključne reči:
nepce, rascep; dijagnoza; histološke tehnike; novorođenče; nazofarinks, neoplazme; polipi; hirurgija, otorinolaringološka, procedure.

Introduction

Hairy polyp is a rare malformation which consists of mesodermal and ectodermal elements, such as adipose, glandular and muscular tissues, covered by the epithelial squamous stratified tissue [1, 2]. A polyp is composed of the histologically mature tissue that is located in an area where it is not typically found. Although its classification and origin are the subject of constant debate, the authors agree that it is a benign lesion without the possibility of malignant alteration,

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with a limited potential for growth [2, 3]. Hairy polyp has been reported in the nasopharynx, soft and hard palate, tongue, oropharynx, tonsil, palatopharyngeus and palatoglossus muscles, external auditory canal, middle ear, mastoid, hypopharynx, oesophagus and trachea [4]. It is the most common congenital nasopharyngeal tumour [5].

Hairy polyp is more often seen in females, mostly in newborns, rarely in adults [2, 6].

The clinical symptoms depend on the size and localization of the mass. It is typically presented by polyhydramnios in the prenatal period and by shortness of breath, stridor and problem with swallowing soon after the birth.

Early surgical excision results in permanent cure [3, 5].

Case report

The female child was born as a second twin from regularly controlled pregnancy. Prenatally, in the 24th week of gestation, *epulis gigantocellularis* was suspected, the magnetic resonance imaging (MRI) was done and a protruding mass was detected, arising probably from the gingiva of alveolar part of the maxilla. During pregnancy, the polyhydramnios was detected. The child was born in the 33th gestational week by a caesarean section due to premature contractions of the mother. Its birth weight was 1,720 g, the Apgar score 3/6. Immediately after the birth, a tissue formation connected with the hard palate was observed, protruding out of the mouth, sized 9 × 3 cm, as well as the split of soft palate and tongue. In the floor of the oral cavity cartilaginous-skeletal-fibrous tumefaction was detected. Other anomalies were not noticed.

Fig. 1 – Intubated newborn with the hairy polyp.

In the first day of life, the child was intubated and the mechanical ventilatory support and nutrition through a nasogastric tube started (Figure 1). Computed tomography of the head and neck was done: the soft tissue formation was detected; it originated from the connection of the middle and the last third of the roof of the oral cavity, protruding out of the mouth till the lower edge of mandible. A skeletal malformation sized 13.5 mm was detected on the left side of the bottom of the oral cavity, originating from the dental alveoli of the second and third tooth protruding medially and forward basis of the tongue. Karyotype was normal. In the fourth day of life, tracheostomy was performed, and until the definitive operative treatment, the mechanical ventilation support was continued (Figure 2).

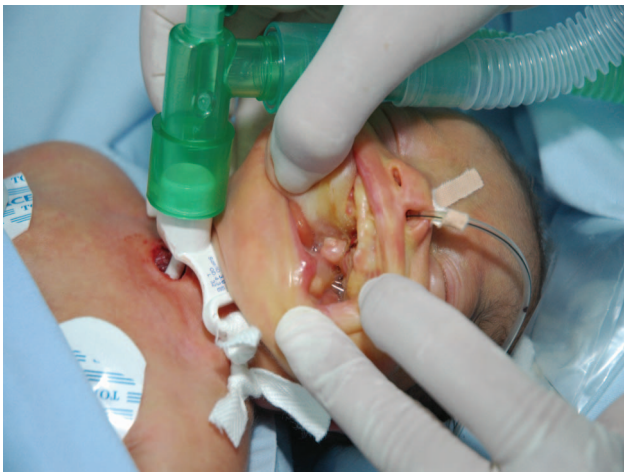

Fig. 2 – Newborn with the hairy polyp after a tracheostomy.

The child developed the clinical and radiological signs of respiratory distress syndrome. In the third week of life, seizures occurred, so an anticonvulsant therapy (phenobarbital) was introduced. The antimicrobial therapy during hospitalisation was conducted according to the antibiogram (ceftazidime, vancomycin). In addition to inhaled corticosteroids, in order to prevent a chronic lung disease, parenteral corticosteroid (dexamethasone) was introduced. In the 40th day of life, a surgery was performed, the tumor mass was entirely excised with the reconstruction of the existing deformity of the mouth (unilateral mandibular osteotomy). The material was sent for a histopathological analysis. The result was a hairy polyp with stratified squamous epithelium on the surface with hair follicles, sebaceous and sweat glands, as well as lobular mature fatty tissue in the deeper layer (Figures 3 and 4).

Upcoming 7 days, the child was on the mechanical ventilatory support, and then she was separated from the ventilator and furthermore was on the oxygen therapy.

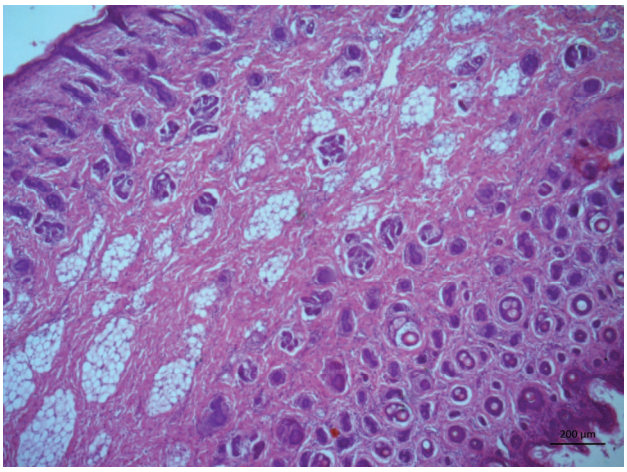

Fig. 3 – Photomicrograph of the hairy polyp shows a lining of stratified squamous epithelium with hair follicles, sebaceous and sweat glands and fatty tissue (HE, ×50).

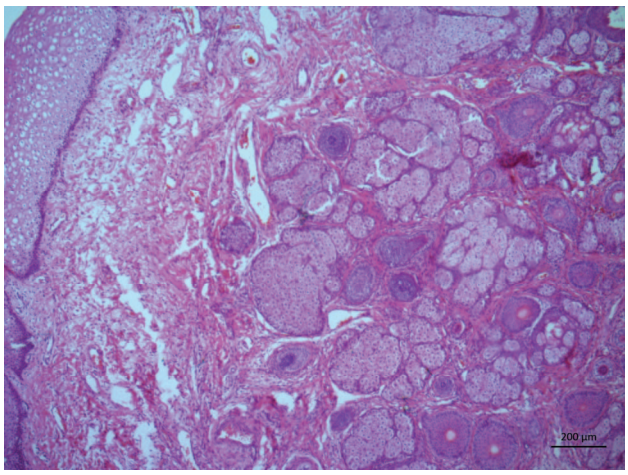

Fig. 4 – Photomicrograph of hair follicles and sebaceous glands of hairy polyp (HE, ×50).

The further course of treatment was complicated by the appearance of pulmonary infection and sepsis, so the adequate antimicrobial therapy (ceftazidime, ciprofloxacin and amikacin) was implemented. Because of this, the child was hospitalized during the first six months of life. In the fifth month of life, a percutaneous endoscopic gastrostomy was placed, so further feeding was conducted this way. Considering the fact that during the whole hospitalization period the child was on oxygen therapy, in the sixth month of life, she was released from hospital with the oxygen concentrator. Later on, there was no sign of residual tumor. Because of the postoperative cicatrization and presence of cleft palate and tongue, upon the advice of the plastic surgeon, palatoplastic was planned after the age of one year.

At the beginning of the second year, the split of tongue and soft palate was sewn. With the establishment of normal swallowing, decannulation and closing of tracheostomy were performed (Figure 5), and then gastrostomy was closed.

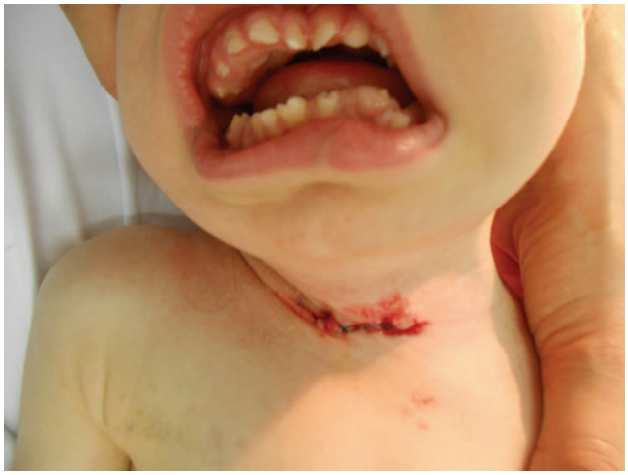

Fig. 5 – The child, 2 years after corrective operation and closing of tracheostomy.

Discussion

Hairy polyp is a rare benign tumor, with an incidence 1:40,000 births [2]. In the published cases there is a distinct female preponderance, 6:1, however, there is no evidence of any genetic inheritance [3]. The presented case underlines the predominance of females. Hairy polyp is usually localized in the nasopharynx, often arising from the soft palate and lateral pharyngeal wall [4, 5]. In our patient, the tumor was arising from the hard palate. Macroscopically, the tumor usually presents as a sausage or pear-shaped, pedunculated mass, with the size which can range between 0.5 and 6 cm [1, 4]. In our patient size of the tumor was bigger than usual – 9 × 3 cm.

There is not a consistent classification of hairy polyps in the literature [1]. Histological examination is crucial in differentiating a hairy polyp from other lesions, such as teratomas, hamartomas, dermoids and choristomas. A hairy polyp is made of tissue of mesodermal and ectodermal origin. The mesodermal components can be fibroadipose tissue, muscle and cartilage. The ectodermal components can be mature stratified squamous epithelium with skin appendages [4]. Unlike hairy polyps, teratomas have the tissue from all three germinal cell layers, they occur equally in males and females and malignant transformation is possible. Hamartomas consist of excessive histologically normal tissue for a particular localisation. Since the pharynx does not contain stratified squamous epithelium, hairy polyps cannot be classified to this group. Dermoids are cystic lesions that contain the desquamated epithelial products. Polyps do not include the ectodermal inclusion cysts within its mesodermal core [1, 4, 7]. According to Arnold [8] , a classification from 1870, hairy polyp is classified as dermoid. A choristoma is a mass consisting of a histologically normal tissue in an anatomically abnormal location. According to some authors, hairy polyps are included into this group [1, 4, 7–10]. The terms as bigerminal choristomas, choristomatous hairy polyps, hairy teratoid polyps, nasopharyngeal dermoids are used in the literature [7, 10, 11].

In our case, the ectodermal elements such as stratified squamous epithelium with skin appendages, as well as the mesodermal structures in the form of adipose tissue were described histologically.

The origin of hairy polyps remains unclear and it is questionable whether they belong to the developmental malformations, or primitive teratomas[7]. There are several proposed theories for its embryogenesis: (1) escape of pluripotent tissue from normal control mechanisms before the 4th week of gestation, disturbed development during the fusion of the epiblast of the stomodeum with the anterior foregut and failure in closure of the 2nd pharyngeal cleft, (2) failure of the nasopharyngeal membrane to regress during the 7th week of gestation, (3) parasitic fetus, derived from the misdirected pluripotent cells that have bypassed the influences appropriate for the local environment, (4) the first pharyngeal apparatus in germ cell rests, (5) inclusion dermoid cyst between two germ layers of the 1st and 2nd branchial arches, (6) inclusion errors in the fusion of the lateral palatal process during the 10th week of embryogenesis, (7) accessory auricles arising from the 1st pharyngeal arch, (8) choristomatous developmental anomalies originating from the 1st branchial cleft area, (9) escape of pluripotential tissue as a disorganized mass, and (10), developmental malformations

related to the development of 1st and 2nd pharyngeal arches [7, 9, 10].

As additional diagnostic procedure, a cytogenetic studies could be done to determine the germ cell origin of a neoplasm of the head and neck. Malignant tumors frequently carry a characteristic chromosomal gain of 12 p, while benign tumors (mature teratomas) show no chromosomal abnormalities [12].

In 10% of cases, the hairy polyps are associated with other first, or second branchial arch malformations, and in another 10% of cases, they are associated with a cleft palate, since the existence of the polyp may interfere with the closure of the palatal shelves [1, 13]. The association of hairy polyps with various congenital malformations has been described, such as cleft lip and palate, facial hemihypertrophy, agenesis of the uvula, external auricle, left carotid artery atresia, ankyloglossia and osteopetrosis. The cleft palate and tongue was detected in our patient.

Polyhydramnios is usually observed during pregnancy because the existence of a polyp disables adequate swallowing mechanism of the foetus [3]. This was also noted in our case. A polyp may partially, or completely obstruct the way to the trachea or oesophagus giving the symptoms of the respiratory or gastrointestinal tract [14–16]. Depending on the location and size, polyps can give symptoms immediately after birth, but they may also accidentally be discovered in late childhood and exceptionally in adults [1, 7]. The most common symptoms at birth are asphyxia, respiratory distress, stridor, cyanosis, feeding difficulties, hypersalivation and recurrent cough. They can also be presented by dysfunction of the Eustachian tube, obstructive sleep apnea or snoring, haemoptysis and persistent nasal secretion [3, 7, 16]. Most of these symptoms were also recorded in our patient. It must be noted that, apart from the presence of pharyngeal mass, the premature birth of our patient certainly contributed to the expression of these symptoms.

A hairy polyp might be overlooked during the endotracheal intubation, because it is usually mobile, soft and pedunculated [3]. Small hairy polyps may be lethal because of delayed diagnosis. To locate small hairy polyps, the physicians should not hesitate to perform further examination because there is the possibility of oversight with only physical examination [17]. Radiological investigations are important to determine the size and location of the tumor, to exclude intracranial expansion, to differentiate it from other masses, to determine the presence of any other associated anomalies and to plan a surgical treatment [4]. For these reasons, in our patient, the computed tomography was performed immediately after birth and it determined the size and location of the tumor mass.

The differential diagnosis of a neonatal nasopharyngeal mass includes teratoma, craniopharyngioma, meningoencephalocele, nasal glioma, neuroblastoma, haemangioma, rhabdomyosarcomas, thymic-thyroglossal or a lingual cyst [1, 3, 4, 11].

A prenatal diagnosis of nasopharyngeal masses is uncommon. If a prenatal ultrasound does show a head and neck mass, the likelihood of perinatal airway obstruction is high. This finding should alert the physician in charge to the potential risk and appropriate prenatal planning should occur [11]. Perinatal management of oropharyngeal masses involves either intrapartum intubation [*ex utero* intrapartum treatment (EXIT procedure)], or resection of the tumor at the time of the cesarean section and prior to cutting the umbilical cord [operation on placental support (OOPS)] [18]. The EXIT procedure can be done in cases where difficulty is anticipated in neonatal airway establishment at delivery, and is done at the time of caesarean section. The partially delivered fetus is maintained on placental circulation while airway is established [19, 20]. *In utero* resection of an oral mass via operative fetoscopy is also one of the therapeutic possibilities [18].

The treatment of choice for this type of tumor is a surgical removal. No recurrence has been reported after complete excision [4]. By monitoring our patient, we confirmed the previous statement. In some published cases the autoamputation of the tumor is described, which resulted in a complete recovery [3, 21].

Conclusion

Although the hairy polyp is a rare tumor, it must be included in the differential diagnosis of pharyngeal mass in the neonatal period. Its presence must be considered in case of breathing and feeding difficulties in infants. The radiological examination can help us in a quick and clear orientation and preparation for the surgery, which should be performed as soon as possible. The final diagnosis is made by obtaining the histological findings. If there are no associated anomalies, and if complete surgical resection of the tumor is performed, further course of the treatment will be favourable.

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Received on October 16, 2017.
Accepted on November 09, 2017.
Online First November, 2017.

CASE REPORT

UDC: 616-006:618
https://doi.org/10.2298/VSP170314177D

Fatal outcome in the patient with the suspected ectopic pregnancy and diagnosed epithelioid trophoblastic tumor

Fatalan ishod sumnjive ektopične trudnoće kod bolesnice sa konačnom dijagnozom epiteloidnog trofoblastnog tumora

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Abstract

Introduction. Epithelioid trophoblastic tumor (ETT) is an extremely rare form and unusual type of trophoblastic tumor. In 1998 ETT became an independent entity because it possesses specific histological and immunohistochemical features which make it different from placental site tumor and choriocarcinoma. ETT origins from intermediate trophoblast and it can overlap with the squamous cell carcinoma as per differential diagnosis. The relevant literature data suggest that surgical treatment is a treatment of choice for ETT considering that its response to chemotherapy is considerably poor. **Case report.** A 35-years-old patient G3 P2 came to an examination due to the low pelvic pain and absence of menstrual cycle. She reported that menstrual cycles were irregular during the past year. During the bimanual pelvic examination, a painful tumefaction being approximately 8 cm was palpated in the pouch of Douglas. The patient was operated on as being suspected for ectopic pregnancy when it was noticed that the aforementioned tumefaction was located retroperitoneally immediately against rectosigmoid part of the colon. After the histopathological analysis of the sample, in order to make the final diagnosis, the immunohistochemical test was performed and it found that this ETT. Due to an inadequate response to administered chemotherapy, both resection of rectosigmoid colon with appurtenant tumor and left hepatectomy with salpingectomy on both sides were performed considering a possibility of gestational trophoblastic neoplasia (GTN) after ectopic pregnancy. Because of dehiscence of colorectal anastomosis, relaparotomy with colostomy bag fitting was performed. The patient died on the day 40 after surgical treatment due to hepatic insufficiency. **Conclusion.** In order to make as much adequate treatment method of ETT as possible, it is necessary to organize a team work with multidisciplinary approach. Surgical resection of the tumor is a primary method for treating ETT.

Key words:
diagnosis; histological techniques; postoperative complications; pregnancy, ectopic; surgical procedures, operative; treatment outcome; trophoblastic neoplasms.

Apstrakt

Uvod. Epiteloidni trofoblastni tumor (ETT) je ekstremno retka forma trofoblastnog tumora. Kao poseban entitet definisan je 1998. godine zahvaljujući specifičnim histološkim i imunohistohemijskim karakteristikama koje su ga činile različitim od tumora placentnog ležišta i horiokarcinoma. ETT potiče od intermedijarnog trofoblasta i može se diferencijalno dijagnostički preklapati sa skvamoznim karcinomom. Podaci iz literature sugerišu da je tretman izbora hirurška resekcija tumora s obzirom na njegovu relativnu rezistentnost na hemioterapiju. **Prikaz bolesnika.** Trideset i pet godina stara pacijentkinja G3 P2 javila se na pregled zbog bolova u maloj karlici i izostanka menstruacije. Bimanuelnim ginekološkim pregledom palpirala se bolno osetljiva tumefakcija promera oko 8 centimetara u Douglasovom prostoru. Pacijentkinja je operisana pod sumnjom da se radi o ektopičnoj trudnoći. U toku operacije uočeno je da se tumefakcija nalazi retroperitonealno neposredno uz rektosigmoidni deo kolona. Nakon biopsije i patohistološke analize uzorka bila je neophodna imunohistohemijska analiza nakon čega je konstatovano da se radi o ETT. S obzirom na neadekvatan odgovor na primenjenu hemioterapiju učinjena je resekcija rektosigmoidnog kolona sa pripadajućim

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tumorom kao i leva hepatektomija uz obostranu salpingektomiju uzimajući u obzir mogućnost primarne lokalizacije gestacijske trofoblastne neoplazije (GTN) nakon ektopične trudnoće. U postoperativnom toku došlo je do dehiscencije kolorektalne anastomoze te je učinjena relaparatomija sa kolostomom. Pacijentkinja je umrla četrdeset dana nakon operacije zbog hepatične insuficijencije. **Zaključak.** U cilju najbolje odluke o metodi lečenja ETT neophodan je timski rad sa multidisciplinarnim pristupom. Primarni metod lečenja ETT je hirurška resekcija.

Ključne reči:
dijagnoza; histološke tehnike; postoperativne komplikacije; trudnoća, ektopična; hirurgija, operativne procedure; lečenje, ishod; neoplazme, trofoblastne.

Introduction

Gestational trophoblastic neoplasia (GTN) implies an invasive mole, choriocarcinoma, placental site tumor and epithelioid trophoblastic tumor (ETT). ETT is an extremely rare form and unusual type of trophoblastic tumor. A term ETT was proposed in 1994 for the first time when it was thought that this kind of tumor occurred as a consequence of administering chemotherapy in the patients with choriocarcinoma, i.e., that chemotherapy influenced the tumor growth by mechanism of drug induced cell changes. In 1998 study by Shih and Kurman [1], it was shown that ETT became an independent entity because it has for some specific histological and immunohistochemical features which make it different from placental site tumor and choriocarcinoma [1–3]. ETT origins from intermediate trophoblast and it can overlap with squamous cell carcinoma as per differential diagnosis considering its common localization in the lower uterine segment or cervix when some cases of ETT were noted in oviducts and *ligamenta lata* [4, 5]. The relevant literature data suggest that a surgical treatment is a treatment of choice for ETT considering that its response to chemotherapy is considerably poor. The ETT affects patients with previous pregnancies during their reproductive period of life. The time between pregnancy and clinical presentation of the disease is from one to eighteen years. Mostly, ETT occurs after term pregnancies. The value of beta-human chronic gonodotropin (β-hCG) at a time when diagnosis is made is increased, but unlike choriocarcinoma, it generally does not exceed about 2.500 mLU/mL [5, 6]. Shih and Kurman [1] reported a rate of metastasis being 25% and a rate of mortality being 10% in ETT. To our knowledge, 94 cases of ETT has been described so far in the literature.

Case report

A 35-years-old patient, G3 P2, was sent by a gynecologist to our institution due to the low pelvic pain and absence of menstrual cycle. For anamnesis, she reported that menstrual cycles were irregular during the past year. During the bimanual pelvic examination, a painful tumefaction being approximately 8 cm was palpated in the pouch of Douglas. An ultrasound view clearly differentiated uterus and both ovaries while the left ovary was located immediately against the aforementioned tumefaction which appeared like hematic mass within the pouch of Douglas. Blood test results were within normal ranges including complete blood count, biochemical parameters and tumor markers (Ca - 125, Ca 19-9, HE-4 and Roma index), except for the increased value of β-hCG which was 198.70 mIU/mL (normal less then 5 mIU/mL). In view of the aforementioned symptomatology which was presented as the low pelvic pain and absence of menstrual cycle as well as an inadequate increase in β-hCG which was 220.01 mIU/mL after 48 h, it was decided to operate the patient in order to explore adnexa. The patient was operated on (on 24 August 2016) as being suspected for ectopic pregnancy when it was noticed that the aforementioned tumefaction was located retroperitoneally immediately against rectosigmoid part of the colon. A surgeon was invited for consultations, a sample was taken for biopsy (*ex tempore* – malignant) and he proposed that further treatment was to be made after obtaining the final histopathological finding and after additional diagnostic procedures. After the pathohistological analysis of the sample, in order to make the final diagnosis, the immunohistochemical test was performed and it was found that this was GTN, i.e., ETT with a suggestion that due to possibility of immunoprofile overlapping, it was necessary to exclude existing of squamous cell carcinoma within anal and genital area. The immunophenotype characteristics of the tumor were the following: panCK+, CK7 focally+, CK18+; CK20-, HPL focally +CEA+/-, p63-/+, p16+, inhibin focally +, beta hCG focally +, OCT3/4-, TTF-, KI67+. Both colposcopic finding and Pap test result were regular. The exploratory curretage exluded presence of GTN within the uterus. The lungs and head were X-rayed, the pelvic and abdominal area were examined with multislice computed tomography (MSCT), and colposcopy was performed. MSCT confirmed both the existance of aforementioned tumefaction in the pelvic area without being clearly differentiated from the surrounding structures and metastatic changes in both liver lobes where the most part of left lobe was changed by metastasis and the size of this change was about 10 cm (Figure 1).

Colonoscopy found polyps in rectosigmoid junction and a sample was taken for a biopsy. X-ray pictures of the head and lungs were regular. After the diagnostic procedures was finished, the International Federation of Gynecology and Obstetrics (FIGO) scores was 11 and the patient was in a high risk group, so multiagent chemotherapy was indicated. Two cycles of cytostatic therapy (methotrexate + cyclophosphamide) were administered to the patient in accordance with a protocol for poor prognosis GTN. The therapy was administered as per the following mode: metotrexate dose of 1 mg/kg, as intramuscular injections on day one, day three, day five and day seven plus folic acid in a dose of 25 to 30 mg on day two, day four, day six and day eight.

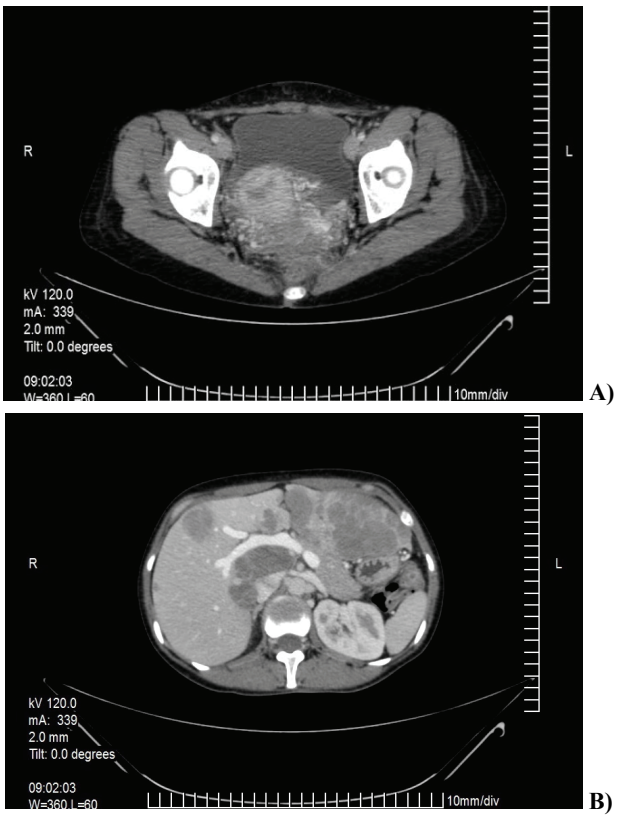

Fig. 1 – A) Tumefaction in pelvic area without clear differentiation from the surrounding structures; B) Metastatic changes in both liver lobes where the most part of left lobe was changed.

Cyclophoshamide dose of 300 mg was administered intravenously. On day seven, after administered chemotherapy, the patient received blood due to severe anemia. Dynamics in the changes of β-hCG values during the treatment did not present any significant deviations from the initial value (at admission 198.70 mIU/mL, at discharge 323.44 mIU/mL). Ultrasonography viewed the tumor mass which dimensions did not change in comparison to the finding prior surgical intervention (Figure 2).

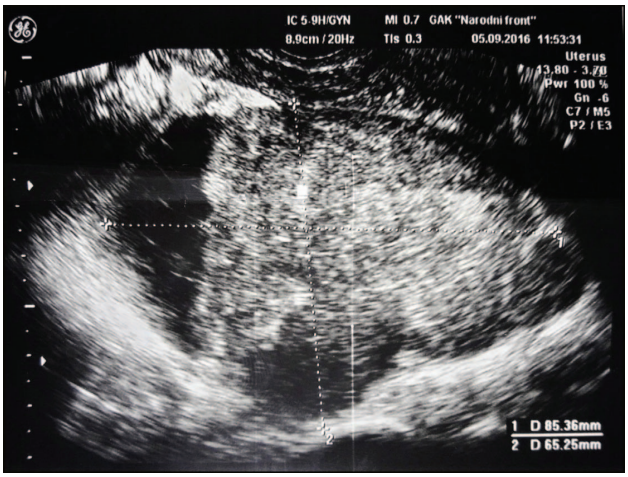

Fig. 2 – Ultrasound image of pelvic mass after chemotherapy revealed left ovary located immediately against the tumefaction (the same as the initial finding).

Because of inadequate response to administered chemotherpy, it was decided that patient was to be subjected to corresponding surgical treatment and in accordance with the localization of the tumor, she was transferred to the Surgery Department of University Medical Centar Bežanijska kosa where both resection of rectosigmoid colon with appurtenant tumor (Figure 3), and left hepatectomy (Figure 4), were performed on October 1, 2016. Figure 5 shows a part of rectosigmoid colon with the tumor and termino-terminal (TT) anastomosis after resection of the tumor.

During this operation, salpingectomy on both sides was performed considering possible primary localization in tubes, i.e., a possibility of GTN after ectopic pregnancy. The right liver lobe was also changed with several minor, individual secondary deposites (Figure 6).

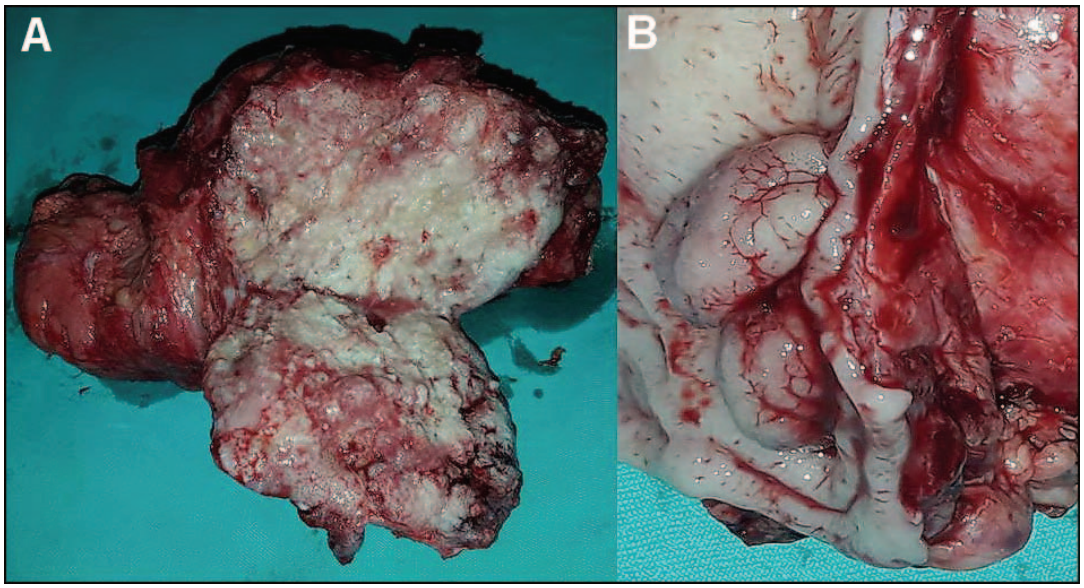

Fig. 3 – A) Macroscopic view of the tumor; B) Polyp which belongs to the resected part of the colon.

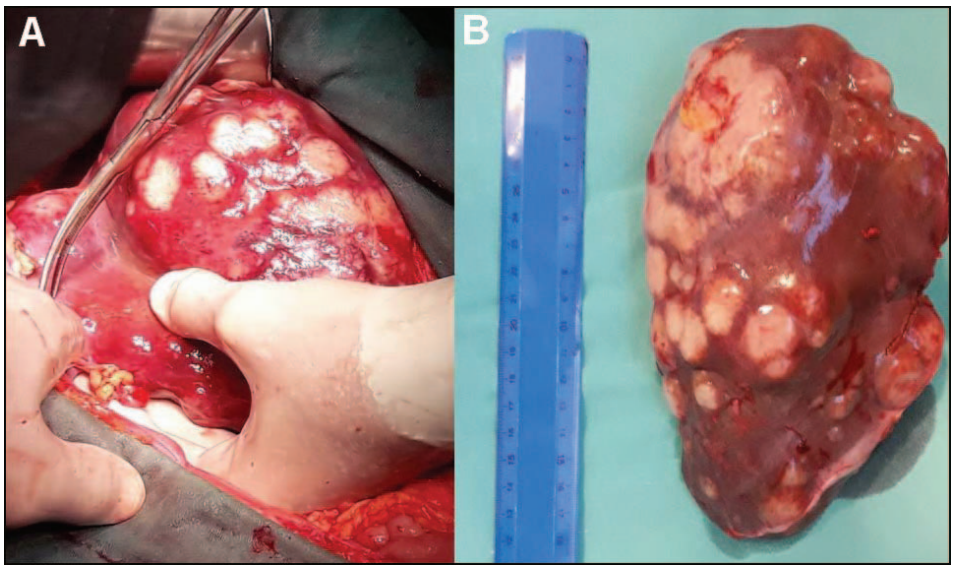

**Fig 4 – A) The left liver lobe changed by metastasis;
B) The sample after the left hepatectomy.**

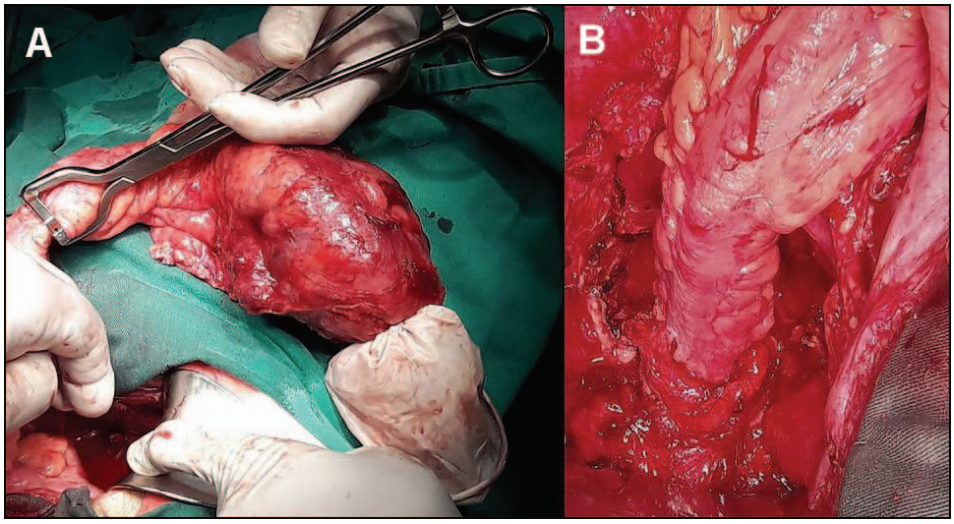

**Fig. 5 – A) A part of rectosigmoid colon with the tumor;
B) Termino-terminal anastamosis after resection of the tumor.**

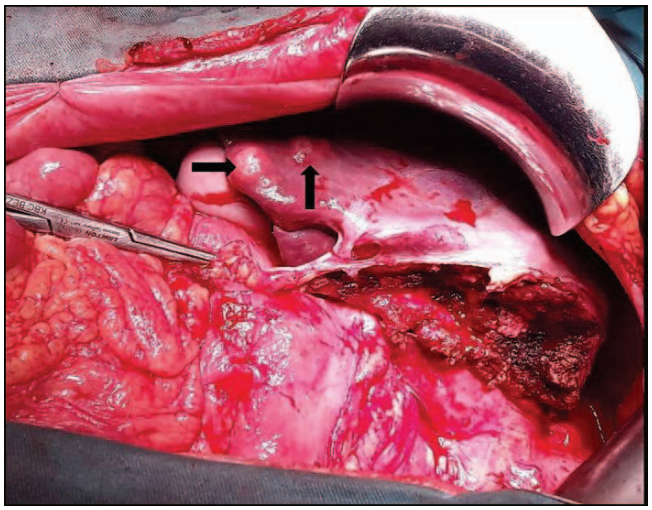

**Fig. 6 – Metastatic changes on the right liver lobe
indicated with black arrows.**

On day 16 after the operation, dehiscence of colorectal anastomosis occured and relaparatomy with colostomy bag fitting was performed. The follow-up values of β-hCG after the surgery did not change significantly and these were 460.80 mIU/mL. Due to the disease expansion, and removal of the left liver lobe presence of due to many secondary deposits, as well as some metastatic changes in the right liver lobe, the patient died on day 40 afer the surgical treatment due to hepatic insufficiency.

Discussion

ETT is a very rare form of trophoblastic tumor which is most often followed by previous term pregnancy, spontaneous miscarriage, ectopic pregnancy and hydatidiform mole, but it is also recorded in nulliparous women. Although it most often occurs at the age group from 15 to 48 years (average 36.1 years), it is also described in the patients in the postmenopausal period [1, 4, 5]. The most common symptoms for this disease are abnormal vaginal bleeding and irregular menstrual cycles [1]. Regarding the usage of ultrasonography in making a diagnosis, there are no specific guidelines which would be used as a basis for suspecting this type of the tumor [7, 8]. Uterus is the most common primary location of ETT (40%) as well as endocervix (31%). The extrauterine location of ETT is rare, and it is often very hard to determine a primary origin of the tumor [6, 9]. ETT most often spreads into the lungs, but also into some other tissues such as brain, liver, pelvic lymph nodes [10]. In our case, this was a very rare retroperitoneal tumor location without the proved primary origin. The fractional exploration curettage excluded presence of the tumor in the uterus and the histopathological finding after salpingectomy of both sides did not confirm possible primary tubular localization. After presurgery chemotherapy had been administered, there was no any improvement, i.e., β-hCG kept its values and the tumor size did not change. Considering resistance of the tumor to the administered treatment, both resection of rectosigmoid colon with the tumor and the resection of the left liver lobe which was completely changed with the tumor, were decided to be done. It was planned to continue with the cytostatic therapy after surgical treatment and the tumor mass reduction. In general, the patients should have one, or two cycles after lowering the β-hCG values to the normal values. Radiotherapy is also possible, while resection of metastasis is recommended for all cases when feasible [11].

Conclusion

The ETT is a rare disease which do not have established treatment protocols. In order to make as much adequate decision on a treatment method as possible, it is necessary to organize a team work with multidisciplinary approach. Despite the fact that the application of diagnostics could have been faster and more precise and that the applied treatment, primarily surgical, was awaited for some time, we believe that this would not have any influence on the treatment outcome considering the fact that it was the late stage of the disease. A surgical resection of the tumor is a primary method for treating ETT. There is neither any standard nor effective chemotherapy protocol due to very small number of cases. Therapy mode varies greatly, and chemotherapy can be administered before and after surgical treatment.

Acknowledgement

This paper is a part of the Project supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, No. 175082.

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Received on March 14, 2017.
Revised on October 17, 2017.
Accepted on November 14, 2017.
Online First November, 2017.

CASE REPORT

UDC: 616.13-007.64:[616.12+616.24
https://doi.org/10.2298/VSP170705179M

Takotsubo cardiomyopathy in aneurysmal subarachnoid hemorrhage – a case report

Takotsubo kardiomiopatija kao posledica aneurizmatskog subarahnoidalnog krvarenja

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Abstract

Introduction. Subarachnoid haemorrhage (SAH) can be followed by cardiac abnormalities. We describe a patient with Takotsubo cardiomyopathy and neurogenic pulmonary edema (NPE) after aneurysmal SAH. **Case report.** A previously healthy, postmenopausal woman, suffered from aneurysmal SAH with consequent hydrocephalus. After external ventricular drainage, craniotomy and clipping of the posterior inferior cerebellar artery aneurysm, the patient developed acute heart failure and NPE. Transthoracic echocardiogram showed the left ventricular apical ballooning and hypercontractile basal segments. On chest radiography, bilateral pulmonary infiltrates were seen. Seventeen days after the SAH attack, the patient was discharged from hospital. Postponed coronary angiography revealed no signs of coronary artery disease. **Conclusion.** This case and review of the relevant literature suggest that Takotsubo cardiomyopathy and neurogenic pulmonary edema are not uncommon after aneurysmal SAH.

Key words:
coronary angiography; diagnosis; echocardiography; pulmonary edema; subarachnoid hemorrhage; takotsubo cardiomiopathy; ventricular function, left.

Apstrakt

Uvod. Subarahnoidalno krvarenje (SAH) može biti praćeno srčanim poremećajima. Prikazali smo bolesnicu kod koje se razvila slika Takotsubo kardiomiopatije i neurogenog plućnog edema, posle ataka SAH. **Prikaz bolesnika.** Prethodno zdravoj osobi ženskog pola, u postmenopauzalnom životnom dobu, dogodilo se akutno SAH je izazvano pucanjem aneutizmatskog proširenja intrakranijalnog, arterijskog krvnog suda praćeno razvojem hidrocefalusa. Posle izvođenja spoljašnje ventrikularne drenaže, kraniotomije i klipsovanja aneurizme na zadnjoj, donjoj malomoždanoj arteriji, kod bolesnice se razvila klinička slika akutne slabosti srčanog mišića i neurogenog edema pluća. Transtorakalni ehokardiogram ukazao je na naduvavanje vršnog dela leve srčane komore i hiperkontraktilnost njenih bazalnih segmenata. Na nativnoj radiografiji pluća viđeni su obostrani, oblačasti infiltrati plućnog parenhima. Posle 17 dana od ataka SAH bolesnica je otpuštana iz bolnice. Naknadna koronarna angiografija nije pokazala znake oboljenja koronarnih arterija. **Zaključak.** Na osnovu kliničke slike bolesnice i uvidom u referentnu literaturu, zaključujemo da se Takotsubo kardiomiopatija i neurogeni edem pluća mogu očekivati sa značajnom verovatnoćom kod bolesnika sa aneurizmatskom SAH.

Ključne reči:
angiografija koronarnih arterija; dijagnoza; ehokardiografija; pluća, edem; krvarenje, subarahnoidno; kardiomiopatija, takotsubo; srce, funkcija leve komore.

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Introduction

Many reports in recent relevant literature emphasize that subarachnoid hemorrhage (SAH) can be followed by cardiac abnormalities [1–7]. The prevalence of SAH-induced neurogenic stunned myocardium varies between 10% and 28% [8, 9]. ECG changes, serum cardiac necrosis markers and wall motion abnormalities have been supposed to be the most common.

Nonetheless, Takotsubo cardiomyopathy (TCM) was casually detected in these patients. The pathophysiology of TCM after SAH is uncertain, but catecholamine release is thought to be the underlying cause in most cases [10–13].

Neurogenic pulmonary edema (NPE) is a clinical syndrome characterized by the acute onset of pulmonary edema following a significant central nervous system insult. In the patients with SAH, reports of NPE incidence range from 2% to 42.9% [14–16].

We described a patient with TCM and NPE after aneurysmal SAH.

Case report

A previously healthy and normotensive 48-year-old female developed progressive loss of consciousness. She was firstly admitted to a regional hospital, with Glasgow Coma Score (GCS) 7 and Hunt & Hess grade 4, and was sedated with midazolam for endotracheal intubation.

Because of suspected cerebrovascular insult, she was transferred to the Emergency Department of University Hospital. On admission, she was unconscious, but sedated, endotracheally intubated, with spontaneous respirations and narrow, symmetric and light-reactive pupils. She had bilateral flexion on rough stimuli.

She was transferred to the computed axial tomography (CAT) scan cabinet. The head CAT scan showed diffuse SAH with blood in the fourth ventrides and ambient cistern, and lateral ventricles, as well as diffuse edema and hydrocephalus – Fisher grade 4 Figures 1A and B, respectively.

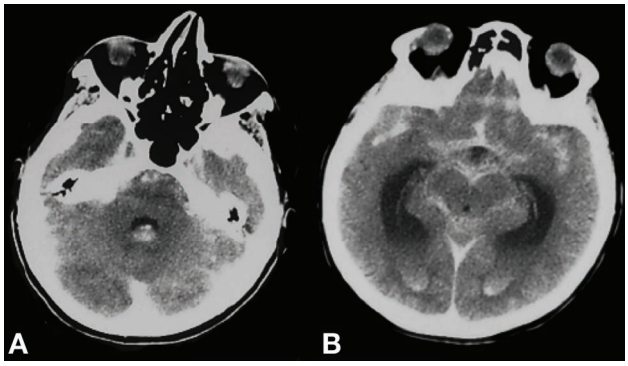

Fig. 1 – The head computed axial tomography scan shows: A) diffuse subarachnoid hemorrhage with blood in the fourth ventricles; B) ambient cistern and lateral ventricles with concomitant hydrocephalus.

She was immediately brought to the operating room for external ventricular drainage (EVD). Ventricular drainage was derived from the frontal horn of the right lateral ventricle. After intervention, the patient was still unconscious and was transferred to the intensive care unit (ICU) for the mechanical ventilation (CPAP, FiO2 40%).

In the ICU, the noninvasive blood pressure (NIBP) measurement was initiated and a central line was inserted for measuring the central venous pressure (CVP). She was hemodynamically stable with NIBP 120 – 130/ 80–85 mmHg, heart rate 72–100 beat per minute (bpm) and CVP 4 cm H_2O.

On the ECG monitor: negative T waves were seen and troponin I was elevated to 2.9 ng mL^{-1} (normal < 0.04 ng mL^{-1}), but the findings were considered as neurogenic stunned myocardium.

Creatine kinase was 146 IU/L and creatin kinase muscle and brain (CK – MB) isoenzyme was slightly elevated to 40 IU/L (normal range: 5–25 IU/L). Intravenous infusion of nimodipine was initiated as well as the routine antibiotic treatment. The patient also got carbamazepine, 2 x 200 mg, per sondam, and 2,500 mL of intravenous crystalloids.

The next day, the patient was awake, successfully disconnected from the mechanical ventilation and extubated. Two days after admission, the digital subtraction angiography of brain blood vessels (Figure 2) and multislice computed tomography (MSCT) angiography with 3-dimensional reconstruction of blood vessels (Figure 3) were done. The diagnosis of the aneurysm of the left posterior inferior cerebellar artery was confirmed.

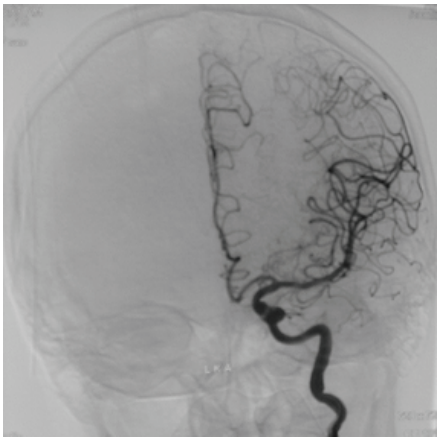

Fig. 2 –Digital subtraction angiography of brain blood vessels reveals an aneurysm of the left posterior inferior cerebellar artery.

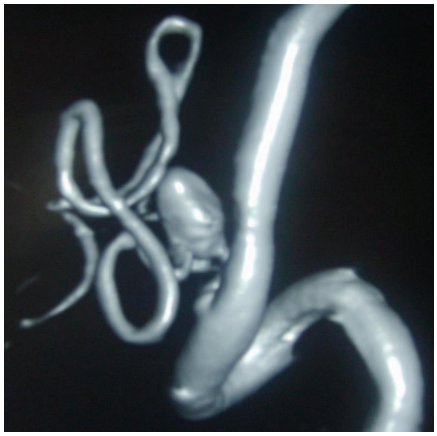

Fig. 3 – Multislice computed tomography angiography with 3-dimensional reconstruction cofirms the aneurysm of the left posterior inferior cerebellar artery.

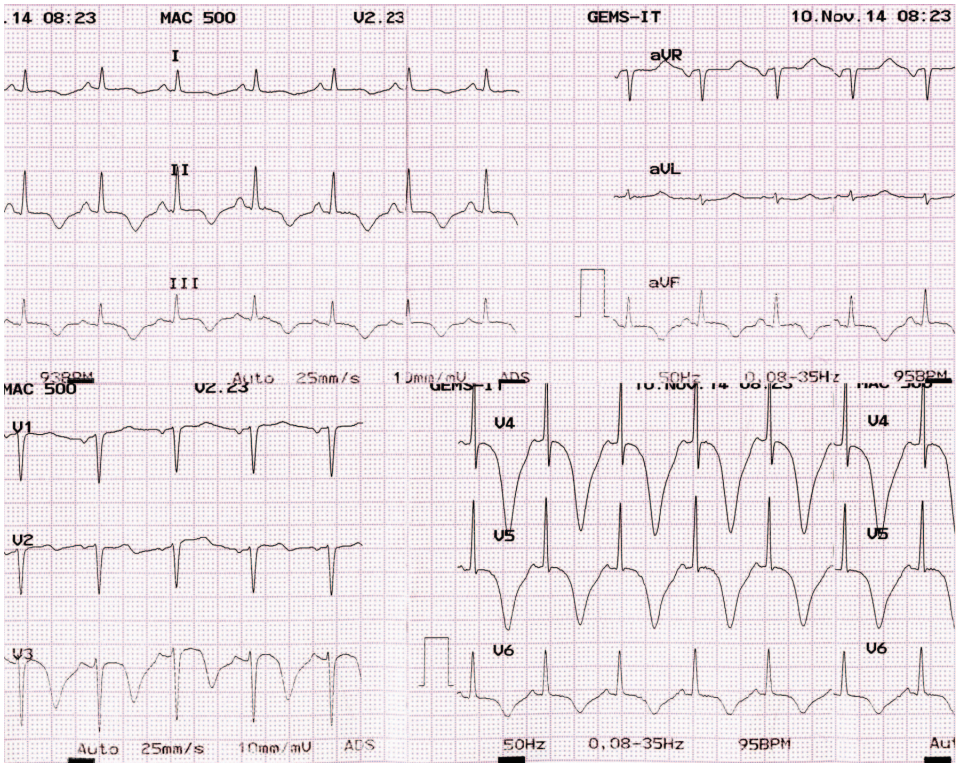

Fig. 4 – The 12-lead electrogardiogram recorded in the patient two days after aneurysmal subarachnoid hemorrhage attack showing deep and negative T-waves in D2, D3, AVF from V3 to V6 leads, as well as a prolonged QTc interval.

The patient was prepared for craniotomy. On chest radiography, the normal findings were described. Deep and negative T-waves were found in: D2, D3, AVF, from V3 to V6 ECG leads as well as prolonged QTc interval (Figure 4).

Troponin I level was lowered to 0.522 ng mL^{-1}. A cardiologist introduced bisoprolol to the therapy. After cardiologist consultation, craniotomy and clipping of aneurism were done, in the general endotracheal anesthesia. It was administered to the patient in a standard manner, and maintained with: remifentanil, sevoflurane and rocuronium in continuous infusion. The patient was extubated on the operating table, eupneic and with no neurological deficit.

Four days after the SAH attack, the patient became tachydyspneic, tachycardic (heart rate 110 bpm) and hypertensive (NIBP 150–160/90–95 mmHg). CVP was 12 cm H_2O. The arterial blood gas analysis showed the following results: pO_2 was 7.9 kPa (normal range 11–14 kPa), pCO_2 4.3 kPa (normal range 4.5–6 kPa), pH 7.48 (normal range 7.35–7.45), SaO_2 92% (normal range 94%–98%), pO2/ FiO2 169 (on the rebreathing mask, FiO2 35%). On the chest auscultation, bilateral rales were heard. During suction through the endotracheal tube, bloody, foamy aspirate was obtained.

She was immediately intubated and assisted with mechanical ventilation (BiLevel mode: with FiO_2 40%, PEEP 4 cm H_2O, peak inspiratory pressure 18 cmH20, pressure support 12 cmH2O and 12 respirations per minute) and midazolam infusion were initiated.

In the repeated arterial blood gas analysis pO2 was 17.3 kPa, pCO2 4.8 kPa, pH 7.51, SaO2 99%, pO2/ FiO2 324. On the chest radiography, bilateral pulmonary infiltrates were seen (Figure 5).

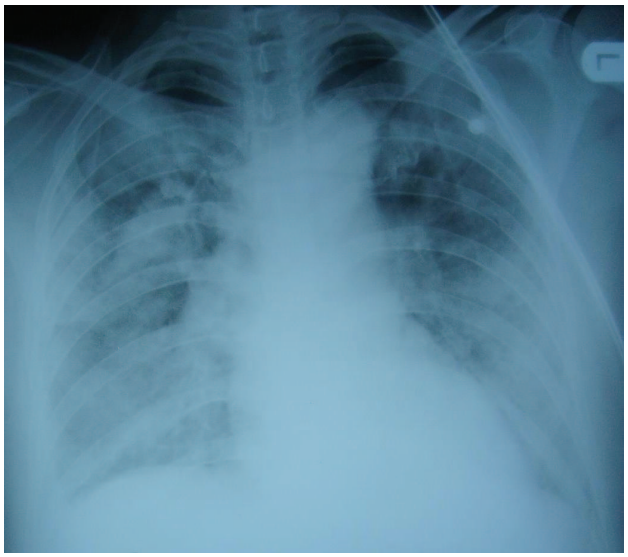

Fig. 5 – A chest x-ray of the patient four days after aneurysmal subarachnoid hemorrhage attack showing bilateral pulmonary infiltrates (neurogenic pulmonary oedema).

The N-terminal pro B-type antriuretic peptide (NT – proBNP) level was 5,829 pg mL^{-1} and troponin I level was 0.59 ng mL^{-1}. In the intensive care unit, transthoracic echocardiogram was made, showing the ballooning of left ventricular (LV) apex and midventricle and hypercontractile basal segments (Figure 6).

The LV diameters in systole and diastole were normal, the ejection fraction (EF) was about 50% and there were no

foreign masses in the apex of LV. Loop diuretic (furosemide 2 x 20 mg, iv.) was initiated as well as low molecular weight heparin – nadroparin 0.4 mL, subcutaneous, x 1, with the permission of a neurosurgeon. Because of high level of C-reactive protein 197/μg/mL, (the normal finding was less than 5 μg/mL) and because of the presence of previously inserted EVD, we decided to start a wide-spectrum antibiotic therapy with meropenem, vancomycin and metronidazole.

At the same time, we took samples of cerebrospinal fluid (CSF) for cytological and biochemical analysis, as well as CSF, blood, urine and tracheal aspirates for microbiological analyses. The analysis of CSF showed no cellular elements with: proteins 1.5 mg mL^{-1} and glucose 3.8 mmol L^{-1}. The CSF culture was sterile as well as the urine culture and blood culture. In the tracheal aspirate, there were 10^3 colonies forming units (CFU) per mL of coagulase negative *Staphylococcus*, sensitive on vancomycin.

Mechanical ventilation was continued till the 8th day after the SAH attack, when weaning from it was done successfully, because of the progressive resolution of pulmonary infiltrates, but without extubation.

A repeated echocardiographic study, 5 days afterwards, showed TCM in regression, with better contractility of the apical LV segment and EF of approximately 60% (Figure 7).

NT-proBNP was 384 pg mL^{-1} and troponin I level was 0.059 ng mL^{-1}. CRP level was 50.2 μg mL^{-1}. Nine days after the SAH attack, the pulmonary infiltrates were completely resolved, so the patient was extubated.

After extubation, the patient was conscious, eupneic, with heart rate 88 bpm, NIBP 130/85 mmHg and CVP 4 cm H_2O. In the arterial blood gas analysis on room air, it was found that pO_2 was 11.6 kPa, pCO_2 5.6 kPa, SaO_2 98%. Two days after, her previously installed EVD was removed in the ICU. The next day, she was discharged from the ICU, and on the 17th day after the insult, she was discharged from hospital. At the time of discharge, she was conscious, eupneic, without the neurological deficit. Postponed coronary angiography revealed no signs of coronary artery disease.

Discussion

The pathophysiology of cardiac dysfunction after SAH is not always clear. The three main theories explaining the pathogenesis of SAH-induced cardiac injury include: multivessel coronary artery spasm causing ischemia, microvascular dysfunction and catecholamine hypothesis.

There is a lack of convincing clinical, or animal data supporting the theory of SAH-induced multivessel coronary artery vasospasm [17]. The clinical data limited to single case reports have failed to demonstrate a decreased perfusion in SAH myocardium [18].

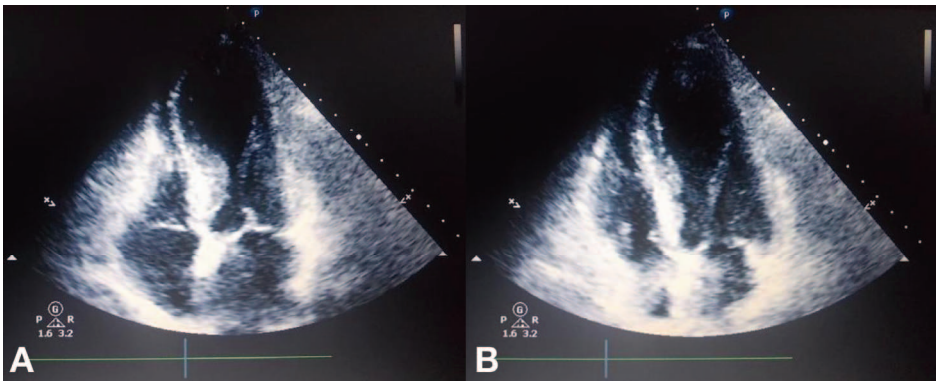

Fig. 6 – Transthoracic echocardiogram (apical 4-chamber view) 5 days after subarachnoid hemorrhage attack showing apical hypokinesia and basal sparing of left ventricle in systole (A) and in diastole (B).

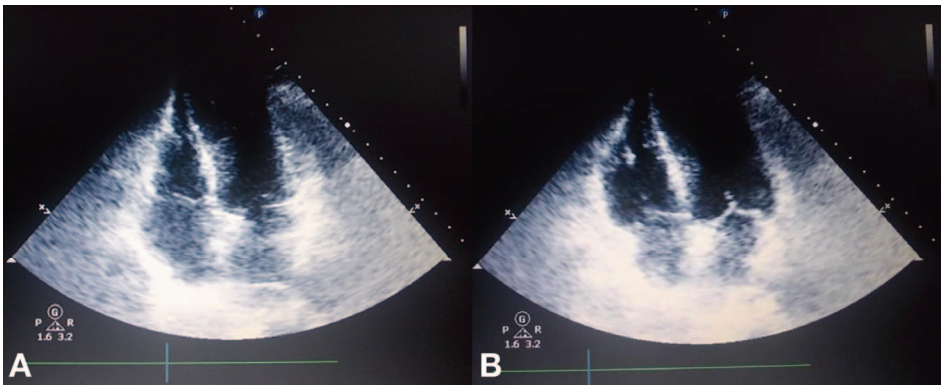

Fig. 7 – Transthoracic echocardiogram (apical 4-chamber view) 9 days after subarachnoid hemorrhage attack showing better apical contractility of left ventricle in systole (A) and in diastole (B).

The most widely accepted theory for the SAH-induced neurogenic myocardial stunning is the "catecholamine hypothesis". This theory suggests that the catecholamine-induced cardiac injury is the underlying cause of cardiac damage in the patients with SAH. Compared with the controls (healthy patients and those with headache), the patients with SAH have an increase in plasma noradrenalin within 48 h after the insult that persists during the first week and normalizes within 6 months [10].

The SAH animal studies were in agreement with the clinical studies [12, 13]. The experimental SAH animal studies not only demonstrate immediate excess sympathetic nervous activation with higher circulating catecholamine concentrations but the heart also appears to be more sensitive to the sympathetic stimulation as well [12, 13, 19]. The local noradrenalin production in the myocardium may surpass the systemic elevation of catecholamines and precipitate global, or regional LV systolic dysfunction [11, 20–22]. An explosive rise in the intracranial pressure (ICP) may cause the sympathetic activation via hypothalamic damage, and therefore an initial transient loss of consciousness at ictus, may represent a risk factor for possible cardiac damage.

Based on previously available data, TCM and neurogenic stunned myocardium appear to be both a marker of the severity of SAH and an independent predictor of symptomatic cerebral vasospasm – both elements associated with worse outcome [9].

NPE is pulmonary edema after the acute neurological insult without underlying lung or heart disease. There are some mechanisms of NPE after SAH.

First, at high pressure, a disruption of the capillary endothelium and alveolar epithelium will occur due to the raised capillary pressure with the development of a high-permeability of blood-lung barrier. A hydrostatic form of NPE develops.

Secondary, a severe depression of the left myocardial function occurring after SAH was regarded as another mechanism involved in NPE pathogenesis, as demonstrated in the retrospective study of 20 patients with NPE [22]. This is evident with most NPE patients demonstrating the increased pulmonary wedge pressure and the reduced cardiac output, or the reduced LV function [6].

Thirdly, some molecules, such as S100B and caspase-1, can be the link between the brain and the lungs that determines the development of NPE after SAH [23, 24].

TCM is a form of neurogenic stunned myocardium which is characterized by the reversible LV regional wall motion abnormalities with a pattern of apical akinesia and concomitant sparing of basal segments. TCM has been reported all over the world and was acknowledged by the American Heart Association as a form of reversible cardiomyopathy.

Four Mayo Clinic diagnostic criteria are required for the diagnosis of TCM: 1) transient left ventricular wall motion abnormalities involving the apical and/or midventricular myocardial segments with wall motion abnormalities extending beyond a single epicardial coronary artery distribution; 2) absence of obstructive epicardial coronary artery disease that could be responsible for the observed wall motion abnormality; 3) ECG abnormalities, such as transient ST-segment elevation and/or diffuse T wave inversion associated with a slight troponin elevation; and 4) the lack of proven pheochromocytoma and myocarditis.

To our knowledge, there are three series of the SAH patients with TCM and few case reports [25–30]. The incidence of TCM in SAH is 0.6%–0.8% [26, 27]. According to Guglin and Novotorova [31] literature reviews in 2011, there were 61 cases of TCM in SAH from 1990.

The first signs of cardiac dysfunction (negative T wave in II lead) in our patient were noticed on admission by ECG monitoring and elevated troponin I. Two days after hemorrhage, the deep negative T-waves in inferior and anterolateral ECG leads and prolonged QTc interval were seen. The patient had no signs and symptoms of acute cardiac disease. Troponin I was lowered, but still elevated as well as CK-MB.

The cardiac abnormalities can be seen with SAH. The ECG changes are present in 50% to 100% of patients, and include the deep T-wave inversion and QTc prolongation. The troponin elevation is seen in 20% to 40% of patients [4, 32, 33]. Elevated troponin I level occurs more frequently in severe SAH, as measured by Hunt and Hess grade, and the peak on the day of ictus with a decay thereafter 5.

Troponin I is 100% sensitive in detecting the LV dysfunction in SAH, compared to CK-MB which is much less sensitive at 29%–60% [4]. The superiority of troponin I over CK-MB as a marker of myocardial injury is consistent with the cardiac literature [34].

BNP and NT-proBNP are another noteworthy serum markers associated with neurogenic stunned myocardium [35]. Elevated plasma BNP is significantly associated with the regional wall motion abnormalities (RWMA), reduced ejection fraction, diastolic dysfunction, pulmonary edema, troponin I elevation, as well as early in-hospital mortality [35, 36].

Because these analyses were no significantly elevated for the diagnosis of acute myocardial infarction, we considered ECG changes as the SAH-induced cardiac injury with no contraindications for craniotomy. After craniotomy, our patient was hemodynamically stable, but the ECG changes persisted. Systolic dysfunction usually develops within the first 2 days after a neurologic event and then recovers [37].

Overall, 10%–28% of patients with SAH had a global or regional LV systolic dysfunction [8]. The development of NPE most frequently occurs within the first week from the beginning of SAH with a peak around day 3. The incidence of NPE decreased with time after SAH. NPE displayed biphasic in the SAH patients, the first peak with cardiogenic NPE caused by a cardiac dysfunction immediately after SAH, and hydrostatic NPE resulted from hypervolemia and low cardiac contractility 7 days after SAH [38].

Four days after hemorrhage, our patient became tachydyspneic, was found hypoxemic, and had to be intubated. Repeated ECG showed decreasing of negative T-waves, but troponin I was almost the same. Furthermore, the physical examination had shown auscultatory bilateral rales and chest radiography showed bilateral pulmonary infiltrates. Our pa-

tient fulfilled the criteria for NPE (physical auscultatory findings, need for oxygenation or mechanical ventilation and bilateral pulmonary infiltrates) [39].

A very high level of NT-proBNP level in blood confirmed cardiac origin of NPE, but our patient had no previous cardiac disease (cardiomyopathy, valvular disease or coronary artery disease). Transthoracic echocardiography clarified our case.

Apical and midventricular hypokinesia of the LV with a basal hypercontractility is a pattern seen in TCM. TCM with the reduced LV function led to congestive heart failure (20%) and pulmonary edema (10%) [15, 40]. The clinical presentation of TCM often resembles acute myocardial infarction, induced by the emotional or physical stress and predominantly occurs in postmenopausal women [41].

The reason behind the striking female predominance (more than 90%) is unclear [42, 43]. The diagnostic features of TCM include the reversible regional wall motion abnormalities beyond a single coronary artery distribution (typically involving the LV apex and midventricle with relative sparing of the basal segment), ECG abnormalities, minor elevation in cardiac biomarkers, and absence of significant coronary artery disease [42–46].

The Mayo Clinic criteria are different in involving midventricle with or without apex, absence of myocarditis and pheochromocytoma and not important role of stress. The cardiac catheterization in the SAH patient is a rare occurrence and should be reserved for the patients with SAH and features incompatible with neurogenic stunned myocardium.

There is no consensus about treatment of TCM. It includes a supportive therapy (intubation and mechanical ventilation, inotropic support, antihypertensives), β-blockers, diuretics, aspirin (if there is coexistent coronary artery disease), low-molecular-weight heparins (if the aneurysm is "solved"), ACE inhibitors [47].

However, the clinicians should be vigilant about potential difficulties that may arise, as the combination of reduced LV function in the setting of cerebral vasospasm window may amplify the deleterious effect of both. This subset of patients may be better treated with inotropic medications during cerebral vasospasm.

As the number of cases of TCM increases, medications for its prevention continue to be investigated. In the animal experiments, α- and β-blockade may be able to prevent TCM [48]. Some clinical data are encouraging. Further prospective studies are warranted to better understand and prevent complications of SAH.

Conclusion

Our case report reminds us that cardiac dysfunction is fairly common after aneurismal SAH and can mimic acute coronary syndrome. Currently, our prevailing practice is to measure the cardiac biomarkers levels in all SAH patients and, so far, to reveal the patients with the risk of regional wall motion abnormalities. Routine transthoracic echocardiography may be necessary in the patients with aneurismal SAH.

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Received on July 5, 2017.
Revised on November 21, 2011.
Accepted on November 23, 2017.
Online First December, 2017.

LETTER TO THE EDITOR/
RESEARCH LETTER

UDC: 579.61:[615.451.1::616.61-78
https://doi.org/10.2298/VSP190913102S

Agrobacterium tumefaciens isolated from hemodialysis water

Agrobacterium tumefaciens izolovan iz rastvora za hemodijalizu

To the Editor

Agrobacterium tumefaciens is Gram-negative, oxidase-positive rod shaped soil bacterium, belonging to the family Rhizobiaceae which includes the nitrogen-fixing legume symbionts. *Agrobacterium tumefaciens* causes a crown gall disease in a wide range of dicotyledonous (broad-leaved) plants, especially members of the rose family and grapevine. This bacterium is known for its remarkable biology, as it is capable to transfer a part of its DNA, known as a tumor inducing plasmid of 200 base pair to the plant, integrating into a plant's genome, and, consequently, causing tumorous changes in plants [1]. Due to its ability to cause such changes in plants it should be considered as a potential pathogen for humans also, especially in an immunosuppressed host [2, 3]. Based on several literature reviews, *Agrobacterium tumefaciens* is isolated occasionally from various human clinical specimens: blood, peritoneal fluid, ascites, catheter or implanted medical devices [4–7].

Recently, during the regular microbiological examination of hemodialysis water used in the Dialysis Center of the Clinical Centre of Vojvodina in Novi Sad, *Agrobacterium tumefaciens* has been isolated.

The microbiological examination was performed according to the ISO 13959:2014, ISO 11663:2014, ISO 23500:2014 standards. Brefly, a sample of hemodialysis water (100 mL) was aseptically filtrated through the membrane filter system (Sartorius, Germany) using the sterile mixed cellulose esters membrane filters 0.45 μm pore size (Millipore, Merck, Germany), after which the membrane filter paper was placed on R2A agar (Oxoid, UK), incubated on 22°C for 7 days. After visible rise of colonies, they were subculturing on blood agar (Oxoid, UK) and further examination and confirmation was done using MALDI-TOF-MS (Matrix-assisted Description/Ionization Time-of-Flight Mass Spectrometry, Brucker, USA). The antimicrobial sensitivity testing of isolated *Agrobacterium tumefaciens* showed its multiple resistance to ampicilin, amoxycilin, gentamicin, trimethoprim-sulfamethoxazole and vankomicin.

The finding of multiresistant *Agrobacterium tumefaciens* in a sample of water for hemodialysis arouses a big doubt about the hygiene of the device for hemodialysis with a potential development of a severe infection in the patients subjected to this treatment. Namely, several scientific papers suggested that *Agrobacterium tumefaciens* was the cause of infections in the patients on hemodialysis [8–10].

To our knowledge, this is the first case of isolation of *Agrobacterium tumefaciens* in a clinical setting in Serbia.

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Received on August 13, 2019.
Accepted on September 16, 2019.
Online First September, 2019.

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b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

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

3. ***Tekst članka***

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

Metode. Jasno opisati izbor metoda posmatranja ili 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.

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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.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp