YU ISSN 0042-8450

# ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

*Часопис лекара и фармацеута Војске Србије*

*Military Medical and Pharmaceutical Journal of Serbia*

# *Vojnosanitetski pregled*

Vojnosanit Pregl 2019; September Vol. 76 (No. 9): p. 855–974.

Vojnosanitetski Pregled 2019 September Vol. 76 (No. 9): p. 855–974.

**vol. 76, br. 9, 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
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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. 9, 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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ORIGINAL ARTICLES / ORIGINALNI RADOVI

Louis Pasteur (December 27, 1822 - September 28, 1895), famous French chemist and microbiologist, is known for the process of food preservation, named after him pasteurization, as well as for the discovery of anthrax and rabies vaccines. The date of his death (September 28) is commemorated worldwide as World Rabies Day with aim to make people aware about the prevention of this horrifying disease and to highlight the progress in fight against it.

Luj Paster (27. decembar, 1822 - 28. septembar, 1895), čuveni francuski hemičar i mikrobiolog, poznat je po postupku konzervisanja hrane, nazvanom po njemu pasterizacija, kao i po otkriću vakcina protiv antraksa i besnila. Datum njegove smrti (28. septembar) obeležava se širom sveta kao Svetski dan protiv besnila sa ciljem da podigne svest ljudi o prevenciji ove zastrašujuće bolesti i istakne napredak u borbi protiv nje.

ORIGINAL ARTICLES


UDC: 616.127-005.8-06
https://doi.org/10.2298/VSP171011163L

# Predictors of health related quality of life three years after myocardial infarction with ST segment elevation

## Prediktori kvaliteta života povezanog sa zdravljem tri godine nakon infarkta miokarda sa elevacijom ST segmenta

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**Institute for Prevention and Cardiovascular Rehabilitation, Niška Banja, Serbia**

**Abstract**

**Background/Aim.** Health-related quality of life (HRQoL) is an important indicator of patient condition following myocardial infarction. It may serve as a predictor of mortality and new hospitalization. The aim of this study was to evaluate the association of selected sociodemographic and clinical characteristics with HRQoL in the Serbian cohort of patients with myocardial infarction with ST segment elevation (STEMI) that were treated with primary percutaneous coronary intervention (pPCI). **Methods.** Patients were recruited from the population of patients with STEMI who were hospitalized in the Clinical Center of Serbia in Belgrade, between 1st December, 2009 and 30th June, 2010. The study was conducted among 507 STEMI patients treated with pPCI. The HRQoL was assessed using the Questionnaire Short Form Health Survey (SF-36). Multivariate logistic regression models were used for each components score in order to determine independent predictors of HRQoL. **Results.** The patients with the lowest tertiles of Physical component score (PCS) and the Mental component summary score (MCS) were older, likely to be females, unpartnered, with a poor economic status, with diabetes, with prior myocardial infarction and with more extensive coronary artery disease. There were more employed and the individuals with smoking history in the group of patients with the higher scores. The characteristics of patients with lower PCS score were: the higher presence of hypertension, prior cerebrovascular insult and left anterior descending artery as infarct artery. This study demonstrated that HRQoL was significantly associated with patient's age, gender, diabetes mellitus, a poor way of living and loneliness. Furthermore, the presence of previous cerebrovascular insult seems to affect the physical component score. **Conclusion.** Knowledge of predictors of HRQoL in the STEMI patients may provide indications for optimal treatment and anticipate their impact on the treatment outcome.

**Key words:**
**st elevation myocardial infarction; health; quality of life; surveys and questionnaires.**

**Apstrakt**

**Uvod/Cilj.** Kvalitet života povezan sa zdravljem (KŽPZ) je važan pokazatelj stanja bolesnika posle preživelog infarkta miokarda i može da posluži kao prediktor mortaliteta i nove hospitalizacije. Cilj studije bio je da se proceni povezanost odabranih sociodemografskih i kliničkih karakteristika sa KŽPZ u grupi bolesnika iz Srbije sa infarktom miokarda sa ST elevacijom (STEMI) koji su tretirani primarnom perkutanom koronarnom intervencijom (pPKI). **Metode.** Bolesnici su regrutovani iz populacije pacijenata sa STEMI koji su bili hospitalizovani u Kliničkom centru Srbije u periodu od 1. decembra 2009. do 30. juna 2010. Studija je sprovedena među 507 STEMI bolesnika koji su tretirani sa pPKI. KŽPZ je procenjen korišćenjem kratke forme upitnika *36-item Short Form Health Survey* (SF-36). Multivarijantni logistički regresioni modeli su korišćeni za svaki kompozitni skor kako bi se utvrdili nezavisni prediktori KŽPZ. **Rezultati.** Bolesnici sa najnižim tercilima Fizički kompozitni skor (PCS) i Mentalni kompozitni skor (MCS) bili su stariji, najčešće ženskog pola, sami, sa lošim ekonomskim statusom, sa dijabetesom, prethodnim infarktom miokarda i ekstenzivnijom koronarnom bolešću. U grupi bolesnika sa višim skorovima, bilo je više zaposlenih i osoba sa istorijom pušenja. Kod bolesnika sa nižim tercilom PCS postojala je veca zastupljenost hipertenzije, prethodnog moždanog udara i leve prednje descendentne arterije kao infarktne arterije. Ova studija je pokazala da je KŽPZ značajno povezan sa starošću bolesnika, polom, lošim ekonomskim statusom, usamljenošću i dijabetesom. Prisustvo prethodnog moždanog udara utiče samo na fizički kompozitni skor. **Zaključak.** Poznavanje prediktora KŽPZ kod STEMI bolesnika može da obezbede indikacije za optimalni lečenje STEMI i da predvidi njihov uticaj na ishod lečenja.

**Ključne reči:**
**infarkt miokarda sa st elevacijom, zdravlje; kvalitet života; ankete i upitnici.**

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## Introduction

Among cardiovascular diseases a myocardial infarction with ST-elevation (STEMI) remains to be a significant cause of morbidity and mortality throughout the world [1, 2]. Conventional treatment is mostly related to extending life, survival and functional outcome. Nevertheless, mortality rates and morbidity are not so valid measures of outcome as they do not reflect all aspects of health [3]. The length of life is considered to be as important as the quality of the additional life years gained by the majority of patients. The priority of today's medicine should be to improve patients' quality and quantity of life [4]. The assessment of health-related quality of life (HRQoL) has become an important part of everyday clinical practice. In the patients with the acute coronary syndrome HRQoL is an important outcome measure because it measures the illness perception instead of the disease itself. HRQoL was recognized to define health from the patients' perspective, in terms of how individuals feel and how they evaluate their health and future prospects [5]. Although there is no universal agreement on what constitutes HRQoL, the current assessment focuses on the domains of social functioning, physical functioning and psychological functioning [6].

There has been little systematic research on quality of life of patients after surviving STEMI. A few studies investigating HRQoL after myocardial infarction mostly focused on the clinical characteristics and treatment procedures, but they failed to take into account the socioeconomic status in their analysis [7, 8]. According to some investigators, the socioeconomic factor seems to have a great impact on HRQoL [9, 10]. The knowledge of predictors of HRQoL in the STEMI patients may provide some indications for further interventions, improve risk stratification in clinical practice and finally lead to the enhancement of secondary prevention. Therefore, the aim of our study was to examine the relationship between the socioeconomic, demographic and clinical parameters and HRQoL three years after surviving STEMI.

## Methods

The study population consisted of 531 consecutive patients admitted to the Clinical Center of Serbia in Belgrade for the primary percutaneous coronary intervention (pPCI) after setting the diagnosis of STEMI between 1st December, 2009 and 30th June, 2010. The patients were contacted three years later in order to fulfill a questionnaire, the 36- Item Short Form Health Survey (SF-36). During a three-year follow-up, 73 patients died (52 male and 21 female). Including deaths, the information on HRQoL for 507 patients was available. Twenty- four patient who were alive and could not be contacted by phone or did not show up for the final examination in order to fill in the questionnaire SF-36 were automatically excluded from the study. Finally, the information about HRQoL was available for 507 patients (including those who died).

The study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Serbia as an obligatory procedure for the PhD thesis.

The diagnosis of STEMI was established and pPCI performed using the guidelines for the management of acute myocardial infarction in the patients presenting with the ST-segment elevation of European Society of Cardiology [11]. In brief, the patients with an episode of chest pain within the last 12 hours and ST-elevation on electrocardiography (ECG) in at least two consecutive leads were included. After pPCI, the patients were hospitalized at the Cardiology Department with continuous clinical, ECG, laboratory and echocardiography monitoring. Echocardiography was performed in the first week of myocardial infarction, on the ultrasonic unit Vivid 4, according to the clinical standard and in accordance with the recommendations related to the current echocardiography guidelines [12].

The data collection was undertaken during the hospitalization period. All recruited patients were interviewed and examined using the standardized methods and instruments. The data was collected regarding demographic, anthropometric, cardiovascular risk factors and medical history prospectively, along with baseline clinical data at the hospital admission.

Three socioeconomic variables were used: education level, living conditions and employment status. The education level was subdivided into two levels: primary, on one hand and secondary and tertiary education on the other. Living conditions were defined using the following question: Considering the monthly resources of your household, how would you say that they allow you to live? Response items "very hardly" and "hardly" were grouped into "poor way of living" and response items "easily" and "very easily" into "good way of living". Also, this study included two other variables: employment on one side, and unemployment and retired on the other.

Age, sex and marital status were used as demographic variables in the analysis. Age was subdivided into two levels less than 70 years and older than 70 years. Marital status was defined as a dichotomous variable: married or other.

The cardiovascular risk factors that were included in the study were history of smoking, diabetes mellitus, hypertension, hyperlipoproteinemia and obesity. Smoking was assessed using two categories: never smoker on one side and previous smoker and active smoker on the other. The diagnosis of hypertension, diabetes and hyperlipoproteinemia was set according to the current guidelines. Obesity was diagnosed if a person had the body mass index more than 30 $m^2$/kg.

Previous cardiovascular events that were included in the analysis were: the previous myocardial infarction, prior cerebrovascular insult and previous revascularization. For all patients who reported such event the medical records were checked up. For the purpose of this study, the parameters for severity of STEMI were included and comprised the following clinical characteristics: infarction localization, infarct artery, extensivity of coronary artery disease and ejection fraction of left ventricle (visually estimated using echocardiography).

*Health status*

To determine HRQoL among the patients with STEMI, the generic questionnaire SF-36 was chosen to be used. The reliability, validity and responsiveness of the SF-36 is well-documented in the patients with coronary artery disease [7]. The SF-36 assesses eight health status domains: Physical functioning, Role physical functioning, Role emotional functioning, Mental health, Vitality, Social functioning, Bodily pain and General health. The scale scores are obtained by summing the items together within a domain. The scoring of eight SF-36 subscales followed the standard procedures and used a 0-100 point scale where 100 is the best and 0 the worst possible score [13]. For the examinees who died, we assumed that they had the worst HRQoL and it was marked 0.

Based on the eight subdomains, the Physical and Mental Component summary scores can be calculated according to an algorithm, with the subdomains Physical functioning, Role physical functioning, Bodily pain and General health being the primary contributors to the Physical component score (PCS) and Role emotional functioning, Vitality, Social functioning, and Mental health being the primary contributors to the Mental component score (MCS) [14].

*Statistical analysis*

The data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 18, SPSS, Inc, Chicago, IL, USA). The continuous variables were expressed as mean ± standard deviation and categorical variables with frequency and percentage. For the comparison of categorical variables, the $\chi^2$ test was used, while the independent Student's *t*-test and one-way ANOVA were used for the continuous variables. The stepwise multivariate logistic regression analysis was performed to determine the independent predictors for HRQoL. Differences were considered significant at the value of $p < 0.05$.

## Results

In the analysis, 393 male patients and 114 female patients were enrolled. The average values of PCS score according to the analyzed variables are shown in Table 1. The significantly lower values of HRQoL in the PCS domains were observed among the all analyzed variables except dislipidemia and obesity. On the contrary, the employed and individuals with smoking history had the significantly higher average values of PCS score (Table 1).

The lower average values of MCS score were recorded among females, individuals older than 70 years, less educated and among individuals with poor way of living. On the contrary, the significantly higher average values of MCS score were recorded among the employed and individuals with smoking history. Among the individuals with diabetes mellitus and hypertension, the lower values of MCS score were recorded. Also, the presence of prior myocardial infarction, prior cerebovascular insult, prior revascularization and the presence of clinical parameters for severity of STEMI led to the significantly lower average values of MCS score (Table 2).

The crude relationship between tertiles of PCS and study variables is given in Table 3. In the group of patients with the lowest PCS score, there were more females, elderly patients, unpartnered ones and individuals with poor economic status. Also, diabetes mellitus was more prevalent among the patients in the lower tertile of PCS, as well as hypertension, prior myocardial infarction and prior cerebrovascular insult (CVI). The presence of clinical parameters for severity of STEMI was more commonly altered among the patients in the lowest PCS tertile. On the other hand, in the group of patients with the highest PCS scores, a significantly higher percentage of the employed and the individuals with smoking history were recorded (Table 3).

According to the data in Table 4, the patients with the lowest tertiles of MCS were older, likely to be females, unpartnered with a poor economic status, with diabetes, with prior myocardial infarction and with more extensive coronary artery disease. On the other hand, there were more employed and the individuals with smoking history in the group of patients with the higher MCS score.

When the multiple logistic regression was applied, it was revealed that being alone, poor way of living, older age, diabetes mellitus and female gender were the independent predictors for the lower PCS and MCS score. The presence of prior cerebrovascular insult turned out to be the independent predictor for the PCS score (Table 5).

## Discussion

This study aims to identify the characteristics of patients with STEMI at the time of their initial hospitalization because of pPCI that were the independent predictors of HRQoL three years after the admission. The functional status was demonstrated to be important, since the higher PCS and MCS were associated with 5%–8% reduction in the risk for hospitalization and 9%–23% reduction in mortality [15]. Furthermore, it was suggested that for optimal treatment of STEMI it was necessary to recognize the patients' differences and their impact on the outcomes of care.

This study revealed that gender, age, socio-economic status and diabetes mellitus were the most important predictors of HRQoL three years after STEMI. It was revealed that female gender was an independent predictor for a poor HRQoL. These results are consistent with the findings of other authors, who had also determined that the gender was an independent predictor for HRQoL in the patients with the acute coronary syndrome. Namely, Pettersen et al. [7] found that two and a half years after the acute coronary syndrome, the female gender was a predictor for worse HRQoL. Furthermore, Mortensen et al. [8] found that after NSTEMI, the women had lower HRQoL than the man, even though they were treated with PCI. These results are in accordance with the findings of Westin et al. [16] and Dias et al. [17] who reported that the female gender was a predictor of worse mental health scores; in contrast, they connected the male gender as a predictor of higher physical health scores.

**Table 1**

**Mean values of Physical component summary (PCS) scores at 36th month follow-up depending on the presence of analyzed baseline variables**

| Variables | n | Mean PCS ± SD | *p* |
|---|---|---|---|
| Gender | | | |
| female | 114 | 34.29 ± 17.92 | 0.001 |
| male | 393 | 38.95 ± 19.03 | |
| Age (years) | | | |
| ≥ 70 | 105 | 28.16 ± 16.46 | < 0.001 |
| < 70 | 402 | 40.44 ± 21.38 | |
| Education | | | |
| primary | 58 | 31.49 ± 18.89 | < 0.001 |
| secondary & university | 449 | 36.97 ± 20.51 | |
| Marital status | | | |
| unapartnered | 44 | 17.87 ± 17.35 | < 0.001 |
| partnered | 463 | 38.99 ± 22.89 | |
| Occupational activity | | | |
| unemployed & retired | 353 | 35.49 ± 17.99 | 0.005 |
| employed | 154 | 41.77 ± 18.54 | |
| Economic status (way of living) | | | |
| poor | 170 | 32.09 ± 17.50 | < 0.001 |
| good | 337 | 42.78 ± 19.72 | |
| History of smoking | | | |
| yes | 391 | 39.69 ± 17.01 | 0.001 |
| no | 116 | 31.88 ± 20.94 | |
| Diabetes mellitus | | | |
| yes | 102 | 29.83 ± 16.80 | < 0.001 |
| no | 405 | 39.93 ± 21.41 | |
| Hypertension | | | |
| yes | 335 | 35.99 ± 16.81 | 0.001 |
| no | 172 | 41.61 ± 18.70 | |
| Dyslipidemia | | | |
| yes | 291 | 39.43 ± 18.90 | 0.028 |
| no | 216 | 35.83 ± 17.64 | |
| Obesity | | | |
| yes | 112 | 39.76 ± 16.45 | 0.265 |
| no | 395 | 37.57 ± 18.57 | |
| Prior MI | | | |
| yes | 62 | 31.98 ± 20.19 | 0.006 |
| no | 445 | 38.72 ± 17.84 | |
| Prior CVI | | | |
| yes | 23 | 25.10 ± 20.57 | 0.001 |
| no | 484 | 38.51 ± 17.94 | |
| Prior revascularizastion | | | |
| yes | 25 | 27.54 ± 22.62 | 0.004 |
| no | 482 | 38.44 ± 17.86 | |
| Infarct localisation | | | |
| anterior | 218 | 35.89 ± 16.81 | 0.031 |
| other | 289 | 39.42 ± 19.88 | |
| Infarct artery | | | |
| LAD | 222 | 35.80 ± 19.94 | 0.022 |
| other | 285 | 39.54 ± 16.68 | |
| Extensivity of CAD | | | |
| multi | 330 | 36.03 ± 16.31 | 0.002 |
| single | 177 | 41.38 ± 18.98 | |
| Ejection fraction (%) | | | |
| ≥ 40 | 438 | 40.12 ± 22.04 | <0.001 |
| < 40 | 69 | 23.79 ± 16.55 | |

**MI – myocardial infarction; CVI – cerebrovascular insult; LAD – left anterior descending artery; CAD – coronary artery disease; SD – standard deviaton.**

**Table 2**
**Mean values of Mental component summary (MCS) scores at 36th month follow-up depending on the presence of analyzed baseline variables**

| Variables | n | Mean MCS ± SD | *p* |
|---|---|---|---|
| Gender | | | |
| female | 114 | 36.56 ± 19.09 | 0.008 |
| male | 393 | 42.08 ± 21.04 | |
| Age (years) | | | |
| ≥ 70 | 105 | 31.87 ± 17.46 | <0.001 |
| < 70 | 402 | 43.16 ± 24.60 | |
| Education | | | |
| primary | 58 | 31.49 ± 9.76 | < 0.001 |
| secondary & university | 449 | 40.50 ± 18.89 | |
| Marital status | | | |
| unpartnered | 44 | 18.52 ± 18.64 | < 0.001 |
| partnered | 363 | 42.06 ± 24.05 | |
| Occupational activity | | | |
| unemployed & retired | 353 | 35.49 ± 17.99 | 0.001 |
| employed | 154 | 41.77 ± 18.54 | |
| Economic status (way of living) | | | |
| poor | 170 | 34.68 ± 18.50 | <0.001 |
| good | 337 | 46.03 ± 10.38 | |
| History of smoking | | | |
| yes | 391 | 42.71 ± 23.06 | <0.001 |
| no | 116 | 34.57 ± 18.15 | |
| Diabetes mellitus | | | |
| yes | 102 | 31.96 ± 17.98 | <0.001 |
| no | 405 | 43.06 ± 23.36 | |
| Hypertension | | | |
| yes | 335 | 39.34 ± 17.87 | 0.017 |
| no | 172 | 43.75 ± 20.38 | |
| Dyslipidemia | | | |
| yes | 291 | 41.56 ± 20.96 | 0.340 |
| no | 216 | 39.87 ± 18.64 | |
| Obesity | | | |
| yes | 112 | 43.59 ± 19.97 | 0.116 |
| no | 395 | 40.28 ± 17.69 | |
| Prior MI | | | |
| yes | 62 | 31.98 ± 17.84 | 0.006 |
| no | 445 | 38.72 ± 20.19 | |
| Prior CVI | | | |
| yes | 23 | 25.10 ± 17.94 | < 0.001 |
| no | 484 | 38.51 ± 20.57 | |
| Prior revascularizastion | | | |
| yes | 25 | 29.20 ± 19.24 | < 0.001 |
| no | 482 | 41.45 ± 24.13 | |
| Infarct localisation | | | |
| anterior | 218 | 38.51 ± 18.30 | 0.020 |
| other | 289 | 42.60 ± 21.14 | |
| Infarct artery | | | |
| LAD | 222 | 38.46 ± 18.21 | 0.016 |
| other | 285 | 42.70 ± 21.17 | |
| Extensivity of CAD | | | |
| multi | 330 | 39.05 ± 17.03 | < 0.001 |
| single | 177 | 44.17 ± 20.74 | |
| Ejection fraction (%) | | | |
| ≥ 40 | 438 | 42.79 ± 25.94 | < 0.001 |
| < 40 | 69 | 28.50 ± 17.74 | |

**MI – myocardial infarction; CVI – cerebrovascular insult; LAD – left anterior descending artery; CAD – coronary artery disease; SD – standard deviation.**

**Table 3**

**Baseline characteristics according to tertiles of Physical component summary (PCS) scores**

| Variables | PCS ≤ 36.6 (n = 163) | 36.7 < PCS > 45 (n = 168) | PCS ≥ 45 (n = 176) | *p* |
|---|---|---|---|---|
| Gender, n (%) | | | | |
| female | 52 (31.90) | 43 (25.60) | 19 (10.80) | 0.002 |
| male | 111 (68.10) | 125 (74.40) | 157 89.20() | |
| Age (years), mean ± SD | 62.46 ± 11.81 | 58.98 ± 10.73 | 55.68 ± 10.60 | 0.104 |
| Education, n (%) | | | | |
| primary | 25 (15.34) | 21 (12.50) | 12 (6.82) | < 0.001 |
| secondary & university | 138 (84.66) | 147 (87.50) | 164 (93.18) | |
| Marital status, n (%) | | | | |
| unpartnered | 22 (13.50) | 12 (7.14) | 10 (5.68) | 0.021 |
| partnered | 141 (86.50) | 156 (92.86) | 166 (94.32) | |
| Occupational activity, n (%) | | | | |
| unemployed & retired | 123 (75.46) | 126 (75.00) | 104 (59.09) | 0.027 |
| employed | 40 (24.54) | 42 (25.00) | 72 (40.91) | |
| Economic status (way of living), n (%) | | | | |
| poor | 73 (44.79) | 57 (33.93) | 41 (23.30) | 0.002 |
| good | 90 (55.21) | 111 (66.07) | 137 (77.84) | |
| History of smoking, n (%) | | | | |
| yes | 113 (69.33) | 131 (77.98) | 147 (83.52) | 0.017 |
| no | 50 (30.67) | 37 (22.02) | 29 (16.48) | |
| Diabetes mellitus, n (%) | | | | |
| yes | 49 (30.06) | 31 (18.45) | 22 (12.5) | 0.001 |
| no | 114 (69.93) | 137 (81.55) | 154 (87.50) | |
| Hypertension, n (%) | | | | |
| yes | 120(73.62) | 116 (69.05) | 99 (56.25) | 0.028 |
| no | 43 (26.38) | 52 (30.95) | 77 (43.75) | |
| Dyslipidemia, n (%) | | | | |
| yes | 85 (52.15) | 96 (57.14) | 110 (62.50) | 0.175 |
| no | 78 (47.85) | 72 (47.17) | 66 (37.50) | |
| Obesity, n (%) | | | | |
| yes | 29 (17.79) | 45 (26.79) | 38 (21.59) | 0.048 |
| no | 134 (82.21) | 123 (73.21) | 138 (78.41) | |
| Prior MI, n (%) | | | | |
| yes | 29 (17.79) | 17 (10.12) | 16 (9.09) | 0.020 |
| no | 134 (82.21) | 151 (89.88) | 160 (90.01) | |
| Prior CVI, n (%) | | | | |
| yes | 14 (8.59) | 5 (2.98) | 4 (2.27) | 0.008 |
| no | 149 (91.41) | 163 (97.02) | 172 (97.73) | |
| Prior revascularizastion, n (%) | | | | |
| yes | 12 (7.36) | 7 (4.17) | 6 (3.41) | 0.133 |
| no | 151 (92.64) | 161 (95.83) | 170 (96.59) | |
| Infarct localization, n (%) | | | | |
| anterior | 79 (48.47) | 64 (38.10) | 75 (42.61) | 0.056 |
| other | 84 (51.53) | 104 (61.90) | 101 (57.39) | |
| Infarct artery, n (%) | | | | |
| LAD | 84 (51.53) | 61 (36.31) | 77 (43.75) | 0.005 |
| other | 79 (48.47) | 107 (63.69) | 99 (56.25) | |
| Extensivity of CAD, n (%) | | | | |
| multi | 120 (73.62) | 109 (64.88) | 101 (57.39) | 0.022 |
| single | 43 (26.38) | 59 (35.12) | 75 (42.61) | |
| Ejection fraction (%), mean ± SD | 44.58 ± 10.53 | 50.89 ± 9.22 | 51.55 ± 8.90 | < 0.001 |

**MI – myocardial infarction; CVI – cerebrovascular insult; LAD – left anterior descending artery; CAD – coronary artery disease; SD – standard deviation.**

**Table 4**

**Baseline characteristics according to tertiles of Mental component summary (MCS) scores**

| Variables | MCS ≤ 39,3 (n = 163) | 39.4 < MCS >52.4 (n = 165) | MCS≥52.5 (n = 179) | *p* |
|---|---|---|---|---|
| Gender, n (%) | | | | |
| female | 55 (33.74) | 34 (20.61) | 25 (13.97) | < 0.001 |
| male | 108 (64.29) | 131 (79.39) | 154 (86.03) | |
| Age (years), mean ± SD | 63.09 ± 11.29 | 57.41 ± 10.11 | 56.48 ± 11.47 | < 0.001 |
| Education, n (%) | | | | |
| primary | 24 (14.29) | 19 (11.52) | 15 (8.38) | 0.188 |
| secondary& university | 139 (82.74) | 146 (88.48) | 164 (91.62) | |
| Marital status, n (%) | | | | |
| unpartnered | 22 (13.50) | 14 (8.48) | 8 (4.47) | 0.029 |
| partnered | 141 (86.50) | 151 (91.52) | 171 (95.53) | |
| Occupational activity, n (%) | | | | |
| unemployed & retired | 124 (76.07) | 124 (75.15) | 105 (58.66) | 0.018 |
| employed | 39 (23.93) | 41 (24.85) | 74 (41.34) | |
| Economic status (way of living), n (%) | | | | |
| poor | 78 (47.90) | 51 (30.91) | 41 (22.91) | < 0.001 |
| good | 85 (52.15) | 114 (69.09) | 138 (77.09) | |
| History of smoking, n (%) | | | | |
| yes | 112 (68.71) | 129 (78.18) | 150 (83.80) | 0.010 |
| no | 51 (31.29) | 36 (21.82) | 29 (16.20) | |
| Diabetes mellitus, n (%) | | | | |
| yes | 50 (30.67) | 26 (15.76) | 26 (14.56) | < 0.001 |
| no | 113 (69.33) | 139 (84.24) | 153 (85.47) | |
| Hypertension, n (%) | | | | |
| yes | 115 (70.55) | 110 (66.67) | 110 (61.45) | 0.223 |
| no | 48 (29.45) | 55 (33.33) | 69 (38.55) | |
| Dyslipidemia, n (%) | | | | |
| yes | 88 (53.99) | 102 (61.82) | 101 (56.42) | 0.148 |
| no | 75 (46.01) | 63 (38.18) | 78 (43.58) | |
| Obesity, n (%) | | | | |
| yes | 30 (18.40) | 39 (23.64) | 43 (24.02) | 0.218 |
| no | 133 (75.46) | 126 (76.36 ) | 136 (75.98) | |
| Prior MI, n (%) | | | | |
| yes | 28 (17.18) | 16 ( 9.70) | 18 (10.06) | 0.030 |
| no | 135 (82.82) | 149 (90.30) | 161 (89.94) | |
| Prior CVI, n (%) | | | | |
| yes | 12 (7.36) | 6 (3.64 ) | 5 (2.79) | 0.068 |
| no | 151 (92.64) | 159 (96.36) | 174 (97.21) | |
| Prior revascularizastion, n (%) | | | | |
| yes | 12 (7.36) | 7 (4.24) | 6 (3.35) | 0.136 |
| no | 151 (92.64) | 158 (95.76) | 173 (96.65) | |
| Infarct localisation, n (%) | | | | |
| anterior | 81 (49.69) | 68 (41.12) | 69 (38.55) | 0.074 |
| other | 82 (50.31) | 97 (58.79) | 110 (61.45) | |
| Infarct artery, n (%) | | | | |
| LAD | 83 (50.92) | 69 (41.82) | 70 (39.11) | 0.057 |
| other | 80 (49.08) | 96 (58.18) | 109 (60.89) | |
| Extensivity of CAD, n (%) | | | | |
| multi | 119 (73.01) | 102 (61.82) | 109 (60.89) | 0.022 |
| single | 44 (26.99) | 63 (38.18) | 70 (39.11) | |
| Ejection fraction(%), mean ± SD | 45.27 ± 10.77 | 50.00 ± 9.02 | 51.76 ± 9.52 | < 0.001 |

**MI – myocardial infarction; CVI – cerebrovascular insult; LAD – left anterior descending artery; CAD – coronary artery disease; SD – standard deviation.**

**Table 5**

**Results of multivariate logistic regression analyses for the association between patient characteristics and Health Related Quality of life (HRQoL)**

| Variables | PCS score | | MCS scores | |
|---|---|---|---|---|
| | OR (95% CI) | *p* | OR (95% CI) | *p* |
| Age (continous) | 1.029 (1.009–1.049) | 0.004 | 1.037 (1.016–1.058) | < 0.001 |
| Female gender | 3.182 (1.280–6.910) | 0.013 | 3.153 (1.268–7.836) | 0.007 |
| Poor way of living | 2.826 (1.229–4.787) | 0.005 | 2.255 (1.411–3.603) | 0.001 |
| Unpartnered | 1.578 (1.011–2.462) | 0.045 | 1.614 (1.063–2.427) | 0.036 |
| Diabetes mellitus | 1.731 (1.055–2.842) | 0.030 | 1.874 (1.134–3.096) | 0.017 |
| Prior CVI | 2.597 (1.08–5.103) | 0.035 | - | - |

**CVI – cerebrovascular insult; PCS – Physical component summary; MCS – Mental component summary; OR – odds ratio; CI – confidence interval.**

The reason for the gender influences on HRQoL in the patients with myocardial infarction is not clear. According to some authors, this gender difference could be explained by the fact that women are increasingly confronted with continuing demands at home and neglect their health needs [9, 18]. On the other hand, according to van Jaarsveld et al. [19] , lower HRQol in women can be explained by higher prevalence of depression among the female patients, limitations of physical and social activity, causing increased escalation of stress and frustration.

Apart from the gender, according to numerous studies, the age plays an important role in HRQoL. Therefore, Jankowska-Polańska et al. [20] in their study indicated that the age of patients negatively affect HRQoL. These findings are in accordance with findings of Beck et al. [21] who also reported older age to be an independent predictor of impaired SF-36 PCS and MCS scores for the population with myocardial infarction who had received PCI. This study confirmed the previous findings, because it was also found that older age was a predictor of poorer HRQoL. The reason why older age negatively influences HRQoL may be explained by the age-related conditions such as frailty limiting the older people physical activity and compounded by other comorbidities that characterize the older population [22].

The presence of risk factors for the coronary artery disease and their impact on HRQoL in the patients with the acute coronary syndrome still remain a controversy. Namely, several studies that dealt with this problem did not relate the presence of risk factors to worse HRQoL [23, 24]. Several publications, on the contrary to these findings, documented that HRQoL became reduced with the presence of risk factors for the coronary artery disease [20]. Also, Dias et al. [17] reported that diabetes, arterial hypertension and loneliness were predictors for the decreased physical component score in the patients with the acute coronary syndrome. Similarly to the finding of Dias et al. [17], this study demonstrates that diabetes mellitus is a predictor for decreased HRQoL.

The explanation for these findings lies in the fact that the presence of these condition may inhibit many normal physical functions; these patients have increased subjective health complains of musculoskeletal, neurological and gastrointestinal pains [25]. Gardner and Montgomery [26] assessed that the patients with this comorbidity had decreased claudication distances, physical function and HRQoL. An impairment of circulation underlines all these conditions and results in pain when physically active, promoting the sedentary lifestyle.

According to some investigators, socio-economic factors seem to have great impact on HRQoL. Therefore, the assessment of the influence of selected socioeconomic variables on HRQoL is an important element of this study. Namely, according to the findings of this study, a low economic status seems to be a strong predictor for impaired HRQOl, because it negatively affected the PCS and MCS. The findings of this study are in accordance with the claims of several other studies that also demonstrated that a poor way of living negatively influences HRQoL [9, 27]. Besides the previously mentioned, our study demonstrated that poor HRQoL was also exhibited by the lonely people. This was also proved by the studies of Jankowska-Polańska et al. [20] and Lane et al. [28] who showed that loneliness turned out to be an independent predictor for HRQoL.

During the last couple of decades, the treatment of STEMI was greatly improved, especially by introducing pPCI, which in turn drastically reduced morbidly and mortality. Previous reports suggested a potential impact of comorbidities on survival after pPCI. These comorbidities are: low ejection fraction, renal insufficiency, Killip class, final thrombolysis in myocardial infarction (TIMI) flow, three vessel disease, and anterior infarction [29, 30]. However, it has not been confirmed if these clinical parameters have an impact on HRQoL. Even if it is to be expected that these parameters may have an impact on HRQoL, this study did not confirm these suppositions because it was found that none of the clinical parameters for severity of STEMI affected HRQoL. Similar to these results are the findings of several other studies that found characteristics related to the severity of myocardial infarction not to be a significant predictor of HRQoL [21, 31]. Beside the previously mentioned, this study demonstrated that the presence of previous cerebrovascular insult negatively affected HRQoL among the patients with STEMI. These findings are in accordance with previous studies that also demonstrated that previous cerebrovascular events had a significant impact on HRQoL [7, 18].

A limitation of this study is that the data represent findings from the group of patients from a single PCI center and not from a multiple different centers. Another limitation is the fact that HRQoL was not measured at baseline at the time of examination of coronary angiography due to the urgency of the procedure. Furthermore, the small size of some variables limits the ability to conduct the stratified analyses. So, the results of this study need to be confirmed in multicenter studies.

## Conclusion

The results of the present study have demonstrated that besides gender and age, diabetes mellitus and some socioeconomic parameters had a significant impact on HRQoL in the patients after surviving STEMI. Apart from the treatment of risk factors for coronary artery disease, a social support may be amenable to interventions and could improve HRQoL in the patients with STEMI. Taking into account differences in age, gender and socioeconomic status may be necessary when planning the intervention strategies (treatment and/or rehabilitation) to improve the effectiveness of secondary prevention.

## Acknowledgments

The author wishes to thank all patients who participated in this study and to all staff of the Clinical Centre of Serbia who helped in collecting and processing data.

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Received on October 11, 2017.
Accepted on October 31, 2017.
Online First November, 2017.

ORIGINAL ARTICLE


UDC: 616.89-008.441.3-08:347.471.7
https://doi.org/10.2298/VSP160914169B

# Is it possible to change the psychopathic traits of substance abusers treated in faith-based therapeutic communities – does the length of treatment matter?

## Da li je moguće menjati psihopatska obeležja kod osoba zavisnih od psihoaktivnih supstanci lečenih u verskim terapijskim zajednicama – da li dužina boravka pravi razliku?

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**Abstract**

**Background/Aim.** Faith-based therapeutic communities (FBTCs) have been increasingly employed as a modality in the treatment of substance abuse. Their program influences behavioral, psychological, cognitive and social changes among their beneficiaries. The aim of the study was to evaluate whether the duration of treatment in a FBTC may contribute to changes in the traits that make the four Hare's psychopathy dimensions – Antisocial behavior, Lifestyle, Interpersonal relationships, and Psychopathic affect. Another aim was to assess whether abusers on treatment in a FBTC have more pronounced psychopathic traits compared with subjects with no history of substance abuse. **Methods**. The study included 59 male subjects, of an average age of 29 years, and of different educational levels, who were divided into three groups: substance abusers who had spent one year in the FBTC; substance abusers who had successfully completed a two-year program in the FBTC; and healthy controls with no history of substance abuse. The Psychopathy Assessment Questionnaire (PAQ) was employed to assess the four Hare's dimensions of psychopathy. **Results**. There were statistically significant differences among the groups on the Antisocial Behavior dimension. For this dimension, there were differences among non-abusers and both groups of substance abusers, with non-abusers achieving the lowest average scores. There were no statistically significant differences between two groups of substance abusers in any of the studied dimensions. **Conclusion**. The longer, two-year treatment in the FBTC did not contribute to changes of the psychopathic traits more than the one-year treatment. In addition, subjects with history of substance abuse undergoing treatment in the FBTC had more pronounced psychopathic traits compared with non-abusers.

**Key words:**
**substance-related disorders; faith healing; therapeutic community; psychopathology; antisocial personality disorder; surveys and questionnaires.**

**Apstrakt**

**Uvod/Cilj.** Verske terapijske zajednice se izdvajaju kao sve češći terapijski modalitet u tretmanu osoba zavisnih od psihoaktivnih supstanci (PAS). Svojim programom one utiču na bihevioralnu, psihološku, kognitivnu i socijalnu promenu među svojim korisnicima. Cilj istraživanja bio je da se ispita da li dužina boravka u verskoj terapijskoj zajednici može da doprinese promeni obeležja koje čine četiri Harove dimenzije psihopatije – Antisocijalno ponašanje, Životni stil, Interpersonalni odnosi i Psihopatski afekat. Drugi cilj je bio da se ispita da li grupa ispitanika sa istorijatom zloupotrebe PAS, na tretmanu u verskoj terapijskoj zajednici, ima izraženija obeležja psihopatije u odnosu na grupu koja nikada nije konzumirala PAS. **Metode.** U istraživanju je učestvovalo 59 muških ispitanika, prosečne starosti 29 godina, svih obrazovnih profila, podeljenih u tri grupe: prva grupa ispitanika zavisnih od PAS koji su godinu dana boravili na tretmanu u verskoj terapijskoj zajednici; druga grupa ispitanika koji su završili dvogodišnji tretman u zajednici; treću grupu su činili zdravi dobrovoljci koji nikada nisu zloupotrebljavali PAS. Korišćen je Upitnik za procenu psihopatije koji meri četiri Harove dimenzije psihopatije. **Rezultati.** Dobijene su statistički značajne razlike između ispitivanih grupa na dimenziji Antisocijalno ponašanje. Na datoj dimenziji beleži se razlika između kontrolne grupe zdravih dobrovoljaca i obe grupe zavisnika, kako onih koji se nalaze godinu dana na tretmanu, tako i onih koji su uspešno završili dvogodišnji tretman u

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zajednici. Na ispitanoj dimenziji, kontrolna grupa zdravih osoba ostvarila je prosečno najniže skorove, dok se ni na jednoj od ispitanih dimenzija nije zabeležila statistički značajna razlika između dve grupe koje su boravile na tretmanu. **Zaključak.** Dvogodišnji boravak u verskoj terapijskoj zajednici nije doprineo promeni obeležja psihopatije u odnosu na jednogodišnji boravak u istoj. Ispitanici koji imaju istorijat zloupotrebe PAS i koji se leče u verskoj terapijskoj zajednici imaju izraženija psihopatska obeležja u odnosu na kontrolnu grupu ispitanika koji nisu nikad zloupotrebljavali psihoaktivne supstance.

**Ključne reči:**
**poremećaji izazvani supstancama; lečenje verom; terapijska zajednica; psihopatologija; ličnost, antisocijalni poremećaji; ankete i upitnici**

## Introduction

Faith-based therapeutic communities (FBTCs) have recently been increasingly employed as a modality in the treatment of substance use disorders [1]. However, there are insufficient scientific publications that would clearly confirm successfulness of this type of treatment. Therefore, their role in the treatment process is usually implied on the basis of qualitative analysis of individual cases, which is primarily based on comparison between the traditional substance abuse treatments and faith-based therapeutic communities [2–5]. The increased interests in the treatment approach adopted by religious therapeutic communities presupposes an increased interest in conceptual and methodological issues related to spirituality and religiousness and their implications on the general well-being [6, 7]. Studies dealing with the role of religion and spirituality on recovery of persons with a history of substance abuse showed that religion and spirituality significantly affected behavioral changes in these patients [8], especially after completion of the treatment [9]. In addition, research showed that the highly religious persons tend to abuse psychoactive substances less frequently, compared with those who are less religious, and moreover, that religiousness in general is associated with the decreased substance abuse [1, 10, 11]. The very concept of therapeutic communities in the treatment of substance use disorders is founded on the principle that the community represents an environment where psychoactive substances are not available and where the persons with dependency problems live together in an organized and structured way that promotes changes towards recovery and social reintegration [12]. Specificity of therapeutic communities lies in the way they interpret the problem of dependency – the dependency is not observed as a disorder, but rather as a whole person problem [13]. Therefore, the treatment is directed towards the person, and not the substance [14]. This approach is founded in humanistic psychology, but also in Christianity – emphasizing a person's potential and ability to grow [15]. A person is considered emotionally weak and immature, but with a potential to change in a positive direction. Socially/morally acceptable living includes values such as honesty, responsible concern, dedication, work ethics and consideration of learning as a main value [16]. Recovery is not used in the traditional medical way (i.e., becoming abstinent), but rather as an indication of a more fundamental change in identity and lifestyle [16]. The FBTCs use the main postulates of organization of therapeutic communities which are additionally enriched by promoting religious convictions and lifestyle preached by the given religion, most often Christianity. FBTCs are specific in that they presuppose living of substance abusers under isolated conditions, mainly in countryside locations, where they have very limited and controlled contacts with their family members, for a period of minimum two years. The main role in a FBTC is played by the clergymen, with the principal task of establishing a connection between substance abusers and the lifestyle and moral principles taught by the religion. An important prerequisite for the FBTC's staff is their ability to serve as role models to their beneficiaries. These role models should be able to demonstrate in a concrete way the abstract concepts of behavior that is presented as socially acceptable and desirable.

The community-based treatment is directed towards correction of socially unwelcome forms of behavior, primarily inadequate personality traits, such as psychopathic traits. One of the most prominent researchers in the field, Hare and Neumann [17] describe psychopathy as a construct that comprises a set of interpersonal, affective and behavioral characteristics. These characteristics cover a wide spectrum of narcissistic and antisocial behaviors, including manipulation, lying, impulsivity, search for sensation, and a lack of empathy, guilt and regret. These traits are frequently encountered in persons dependent on psychoactive substances, and are therefore considered the most frequent psychiatric comorbidity seen in therapeutic communities [18–20]. The persistent cognitive, emotional, interpersonal and behavioral problems that characterize this group of disorders, and their prominence, contribute to a poorer outcome in the treatment of substance abuse [21]. It has been frequently suggested that there is still no efficient treatment for correction of psychopathological traits [22–24]. It would be interesting to analyze effects of alternative treatments, such as the FBTC, on the psychopathic traits of substance abusers.

The goal of treatment in the FBTCs is to achieve as successful social reintegration of beneficiaries as possible, which requires mastering socially acceptable behaviors [25]. This primarily means attempting to correct or at least diminish socially unacceptable psychopathic traits. The aim of the research was to evaluate whether the length of treatment in the FBTC is a factor affecting changes in psychopathic traits, i.e., four Hare's dimensions of psychopathy – Antisocial behavior, Lifestyle, Interpersonal relationships and Psychopathic affect. Another aim was to assess whether substance abusers in the FBTC have more pronounced psychopathic traits compared with subjects with no history of substance abuse.

## Methods

The study included 59 males of an average age of 29 years and of different educational levels.

All participants were divided into three groups: the subjects who were on substance abuse treatment in the FBTC for one year (hereafter one-year treatment group; n = 20); the subjects who had successfully completed two-year treatment for substance abuse in the FBTC (hereafter two-year treatment group; n = 20); and the healthy volunteers with no history of substance abuse (hereafter non-abusers; n = 19).

The Psychopathy Assessment Questionnaire (PAQ) [26] comprised 40 items with a binary format of responses. The questionnaire was constructed according to the Cleckley-Hare's criteria by comprising a number of items related to 20 psychopathy traits as defined by Hare. The number of items was reduced on the basis of psychometric indicators and items that differentiate persons with and without psychopathic traits. Factor analysis of baseline items identified four factors corresponding to the four Hare's dimensions [26]. The questionnaire contained four subscales that measured four dimensions, each operationalized with 10 items. The dimension Interpersonal Relationships included items that corresponded with a poor control of aggression, unscrupulousness, belief in own charm and manipulativeness. The dimension Psychopathic affect comprised the indicators of cold-bloodedness, superficial affect and lack of guilt and regret. Lifestyle comprised items that indicated a highly pronounced need for stimulation, irresponsibility and tendency towards substance abuse. Antisocial behavior related to items that indicated the physical aggression, conflicts with law, problematic family relationships in childhood and tendency towards crime. The whole scale had a high confidence level (Cronbach's alpha = 0.79). The PAQ dimensions are expressed as the summary scores. The dimensions were calculated according to a pre-defined key, as described by the authors of the test.

The research was conducted over a period of nine months. The study complied with the World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects.

We had contacted one of Orthodox FBTCs in Vojvodina, which gave a permission for their beneficiaries to participate in the research. The researchers were allowed access to data about the persons who had been on the treatment in the community for one year at the time of the research as well as about those who had successfully completed two-year treatment in the community two months prior to the start of the research. The community's only requirement was that their identity remained anonymous, in terms of their name and location. Public invitation through social media networks was used to recruit subjects from the general population who had no history of substance abuse and were willing to participate in the research. All three groups of subjects signed an informed consent and the subjects' anonymity was guaranteed. The one-year treatment group filled in the questionnaire on the premises of the FBTC. The two-year treatment group and non-abusers completed the questionnaire at their homes.

The treatment in the FBTC was considered successful if a subject had spent two years in the community, adhering to all rules of the community (completed abstinence from substance use, participation in all treatment activities – daily prayers, occupational therapy including physical work, controlled possibility of communication with the family, leaving the FBTC's premises only with a permission). The beneficiaries were allowed to see their family members only after one year of treatment.

The residence in the FBTC was based on a strictly defined structure of daily activities, carried out according to the pre-defined rules of behavior. Daily activities started with getting up and collective chanting (06.30–06.45h), followed by a morning prayer (06.45–07.30h), breakfast (07.30–08.30h), delegation of daily tasks and performance of the tasks (08.30–12h), collective praying with rosaries and chanting, lunch and free activities (12.30–14h), continuation with daily tasks (14–17.30h), daily hygiene (17.30–18.45h), evening prayer and dinner (from 18.45h) and going to bed (22h). During the dinner, the beneficiaries read excerpts from the Gospel for the day (each one interprets messages) and afterwards they discuss the daily tasks, e.g., tasks they had and whether they had performed them successfully. If they had failed to perform the tasks, the beneficiaries are sanctioned, most commonly in the form of being denied a meal or having to perform low bows (prostrate) during a meal or stand at the table during a meal. The daily tasks may include work with animals, farming, workshop tasks, preparation of meals, cleaning the house and doing the laundry. Free/leisure activities included reading, having a walk, listening to music, conversation, writing and singing. The beneficiaries are forbidden to lie in bed during free activities. They also read a part from the Book of Psalms every day. The newcomers were obliged to follow an elder beneficiary, whom they call "angel" and were not permitted to leave him in the first days after their arrival, not even when they went to the toilet. The hierarchical organization within the community presupposed that 3 to 4 persons who spent the longest time in the community were responsible for delegation and supervision of tasks. Telephone communication was allowed only to the oldest and the most reliable beneficiary in the group, and only in the presence of a priest and an educator in charge of the given group. The educators were ex-beneficiaries of the community who had successfully completed the two-year treatment. There were usually between 19 and 25 beneficiaries in a group at a time. Disobedience of the strict structure and rules lead to expulsion from the community.

The gathered data were processed with the statistical package SPSS 19.0. The differences among the groups were compared using the nonparametric statistics (Kruskal-Wallis and Man-Whitney tests). The main sociodemographic data were presented using the descriptive statistics.

## Results

The study included only male subjects. Analysis showed the significant differences among the studied groups in the main sociodemographic characteristics, i.e., age [H (2

n = 59) = 12.78; $p = 0.02$], and educational level ($\chi^2 = 13.44$; df = 6; $p = 0.036$). The average ages of the one-year treatment group, two-year treatment group and non-abusers were 32.00, 29.95 and 27.58 years respectively (Table 1). There was a statistically significant difference between the non-abusers and abusers. The group of non-abusers had a higher average educational level compared with the two-year treatment group. There was no significant statistical difference among the studied groups in marital status ($\chi^2 = 6.68$; df = 4; $p = 0.153$). A majority of subjects were single and completed secondary education. A majority of subjects from the two groups of abusers had been convicted. There was no difference in the type of substance used between the two groups of abusers. The analysis of correlation matrix did not show a significant correlation between the subjects' age and the studied dimensions of psychopathy. On the other hand, we found a significant correlation between education and the psychopathy dimensions. Accordingly, we performed the partialization of effects of education for all applied measures of psychopathy.

Differences on PAQ dimensions in those three groups were analyzed using the Kruskal-Wallis test. The mean ranks are presented in Table 2.

The Kruskal-Wallis ANOVA showed the presence of statistically significant differences between the groups only on one PAQ dimension – Antisocial Behavior (Table 2). In order to determine the degree of difference among the groups, the *post-hoc* Mann-Whitney *U*-test was employed. Because of the multiple comparisons, the Bonferroni correction of alpha values was performed. The Mann-Whitney *U*-test results are presented in Table 3. The significant differences were found for the dimension Antisocial behavior between the two-year treatment group and non-abusers as well as between the one-year treatment group and non-abusers (Table 3). Non-abusers had the lower Antisocial behavior scores (Table 2).

**Table 1**

**Sociodemographic characteristics of study groups**

| Sociodemographic parameters | One-year treatment group (n = 20) | Two-year treatment group (n = 20) | Non-abusers (n = 19) |
|---|---|---|---|
| Education, n (%) | | | |
| primary | 4 (20.00) | 2 (10.00) | 0 (0) |
| secondary | 11 (55.00) | 17 (85.00) | 14 (73.10) |
| 2-yr tertiary | 4 (20.00) | 0 (0.00) | 1 (5.30) |
| tertiary | 1 (5.00) | 1 (5.00) | 4 (21.10) |
| Marital status, n (%) | | | |
| single | 13 (65.00) | 18 (90.00) | 14 (73.10) |
| married | 3 (15.00) | 2 (10.00) | 4 (21.10) |
| divorced | 4 (20.00) | 0 (0.00) | 1 (5.30) |
| Convicted, n (%) | | | |
| yes | 16 (80.00) | 14 (70.00) | - |
| no | 4 (20.00) | 6 (30.00) | - |
| Age (years), mean ± SD | 32.00 ± 4.507 | 29.95 ± 3.517 | 27.58 ± 11.46 |
| minimum-maximum | 26–44 | 26–42 | 19–59 |

**SD – standard deviation**

**Table 2**

**Mean ranks on the PAQ dimensions in three groups of subjects**

| PAQ dimensions | Mean rank | | | H | *p* |
|---|---|---|---|---|---|
| | One-year treatment group | Two-year treatment group | Non-abusers | | |
| Antisocial behavior | 37.38 | 36.03 | 15.89 | 19.13 | 0.000 |
| Lifestyle | 36.63 | 28.93 | 24.16 | 5.25 | 0.072 |
| Psychopathic affect | 31.13 | 25.55 | 33.50 | 2.22 | 0.330 |
| Interpersonal relationships | 34.00 | 25.43 | 30.61 | 2.53 | 0.283 |

**PAQ – Psychopathy Assessment Questionnaire.**

**Table 3**

**Mann-Whitney *U*-test (MW U) of differences among three groups on the Antisocial behavior dimension**

| Dimension | Differences between groups | MW U | z | *p* |
|---|---|---|---|---|
| Antisocial behavior | 1 : 2 | 182.00 | -.487 | 0.626 |
| | 1 : 3 | 60.50 | -3.68 | 0.000 |
| | 2 : 3 | 51.50 | -3.93 | 0.000 |

**Groups: 1 – One-year treatment group; 2 – Two-year treatment group; 3 – Non-abusers.**

## Discussion

In the present study, we used the PAQ questionnaire constructed to measure four Hare's dimensions of psychopathy, with the aim to determine whether the length of treatment of substance abusers in a FBTC may contribute to changes of their psychopathic traits. We found the differences among the study groups in one dimension only – Antisocial Behavior. For this dimension, we found significant differences between the non-abusers, the one-year treatment group and the two-year treatment group. The one-year treatment group had the highest scores on this dimension, which indicates that they had a higher degree of physical aggression, conflict with law, problematic family relationship in childhood and generally more pronounced tendency towards violent behavior compared to the other two groups of subjects. These traits were less pronounced in the group that successfully completed the treatment, although the difference between the one-year and two-year treatment groups was not statistically significant, but inferred from the analysis of mean values. These traits were the least prominent in the non-abuser group. This finding suggests that the two groups of abusers with different lengths of treatment did not differ in psychopathic traits measured by the dimension Antisocial behavior. These findings are in agreement with the previous studies confirming that psychopathic traits were more pronounced and frequent in substance abusers, particularly among the violent offenders [27]. We also found that most of our subjects with a history of substance abuse had been convicted (80% of the one-year treatment group and 70% of the two-year treatment group). Although it was expected that the study groups would have differed on other dimensions of the PAQ, i.e., Lifestyle, Psychopathic affect and Interpersonal relationships, there were no significant differences.

Taking into account that there was no statistically significant difference between the one-year and two-year treatment groups, whereas there was a difference between non-abusers and both groups of abusers, it can be assumed that the pronounced psychopathic traits are permanent personality characteristics, i.e., that regardless of the duration of treatment in the FBCT, it is not possible to alter these traits. These findings are in agreement with a volume of research showing inability of correction of these traits, regardless of the type of treatment [23, 24, 28–32]. However, these findings can partially be explained in the context of the transversal study design, and a future longitudinal study of one-year treatment group could contribute to a clearer picture and more thorough data. On the other hand, the studies suggesting that certain psychopathic behaviors can be corrected related mostly to younger age, i.e., adolescents, which was not the case in our research [32, 33]. The meta analyses dealing with the treatments aimed at correction of psychopathic traits suggested that there was usually no significant improvement and that the corrections should be approached with realistic expectations [34, 35]. It means that we should not expect a complete removal of all socially unacceptable psychopathic traits, but rather the gradual and minor corrections [34, 35]. If we observe the possibility of treatment of psychopathy in this way, the role of length of stay in the FTBC could potentially play a role in this process of change. This is corroborated by our findings that, although statistically insignificant, minor differences did exist between two abusers groups on the PAQ dimension Antisocial behavior. An alternative interpretation may be that although a successfully completed two-year treatment in the FBTC did not lead to correction of psychopathic traits, as the permanent personality traits, it still may contribute to the subjects' mastering a higher degree of control over their socially unacceptable behaviors. This implies that future research in the FBTCs should be directed towards the assessment of control over their own inadequate behavior, rather than change of permanent personality traits. In this case, the instruments used should also be specific for the assessment of control of behavior, and not of personality traits.

A limitation of the present study can be observed in the context of the sample size. Future research should include more participants within all groups, since it is possible that our results were influenced by the sample size. It is also possible that we had "unrealistic" expectations regarding getting statistically significant differences among the groups on all PAQ dimensions. In addition, it would be useful to include and compare different types of FBTCs, besides duration of treatment, in order to evaluate efficacy of their programs. On the other hand, the advantages of the study may be interpreted through the difficulties the researchers encountered. Namely, it was very difficult to form a study sample for this type of research, because the number of persons dependent on psychoactive substance staying in a community and those who successfully completed the FBTC program was very limited. Another advantage of the present study is that it is one of the first studies in the region on the effect of FBTC on the correction of certain psychopathic traits. Considering that religious therapeutic communities represent a closed type of communes, this means that researchers are usually not able to access them. Therefore, the very fact that a religious therapeutic community permitted conducting a study on their premises represents a significant step forward in this research field.

## Conclusion

The longer, two-year treatment in the FBTC did not contribute to changes of the psychopathic traits more than the one-year treatment. In addition, the subjects with a history of substance abuse undergoing the treatment in the FBTC had more pronounced psychopathic traits compared with the non-abusers.

## Acknowledgement

The research was funded by the project of the Ministry of Education, Science and Technological Development of the Republic of Serbia named "Psychological Foundations of Mental Health: Hereditary and Environmental Factors" (ON179006).

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Received on September 14, 2016.
Revised on November 08, 2017.
Accepted on November 14, 2017.
Online First November, 2017.

ORIGINAL ARTICLE

UDC: 617.7
https://doi.org/10.2298/VSP171109185D

# Correlation between conjunctival scraping cytology and other clinical dry eye metrics in determination of dry eye related inflammation

## Korelacija između citološkog nalaza epitela konjunktive dobijenog skrajpingom i drugih kliničkih testova pri utvrđivanju inflamacije suvog oka

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**Abstract**

**Background/Aim.** New and improved definition of dry eye disease (DED) emphasized that hiperosmolarity and inflammation with initial tear film instability play etiological role. The aim of this study was to explore relation of some commonly used clinical tests to dry eye disease (DED) related inflammation measured by conjunctival scraping cytology. **Methods.** We examined 100 subjects, 80 of them having DED. We performed Schirmer without anesthesia (Schirmer I), Fluorescein Tear Break Up Time (FTBUT), Rose Bengal (RB), Lid Parallel Conjunctival Folds (LIPCOF), Tear Meniscus Height (TMH) and Tear Ferning (TF) and compared the values to scraping scores of tarsal conjunctiva. **Results.** FTBUT had the best sensitivity (93.6%). The highest specificity was found with RB (93.2%), but it was also high with Schirmer I, TF and FTBUT (respectively 89.8%, 84.5%, 78.0%). RB and FTBUT had the highest correlation with conjunctival scraping score ($r = 0.707$, $p < 0.001$; $r = -0.507$, $p < 0.001$). **Conclusion.** In our study, FTBUT, though often used in many combinations of the DED tests, showed a remarkably high sensitivity and specificity on its own, as well as good correlation with DED related inflammation detected with conjunctival scraping cytology.

**Key words:**
**dry eye syndromes; conjunctiva; cytological techniques; sensitivity and specificity; diagnostic tests, routine; diagnosis, differential.**

**Apstrakt**

**Uvod/Cilj.** Nova unapređena definicija bolesti suvog oka ističe hiperosmolarnost i upalu sa inicijalnom nestabilnošću suznog omotača kao najčešće etiološke faktore. Cilj rada bio je da se ispita korelacija između nekih često korišćenih kliničkih testova za suvo oko i citološkog nalaza epitela konjuktive dobijenog skrejpingom pri utvrđivanju inflamacije suvog oka. **Metode.** Od ispitanih 100 bolesnika, dijagnozu suvog oka smo postavili kod 80. Učinjeno je merenje sekrecije suza bez anestezije u 5 minuta Schirmer trakom (Schirmer I), vreme prekida suznog filma obojenog fluoresceinom (*Fluorescein Tear Break Up Time* – FTBUT), bojenje površine oka vitalnom bojom Rose Bengal (RB). Ispitano je prisustvo nabora konjunktive paralelnih ivica donjeg kapka (*Lid-Parallel Conjunctival Folds* – LIPCOF), izmerena visina meniskusa suza (*Tear Meniscus Height* – TMH) i urađen test granja suze (*Tear Ferning* – TF). **Rezultati.** FTBUT je pokazao najvišu senzitivnost (93,6%). Najvišu specifičnost je pokazao RB test (93,2%), ali je visoka specifičnost utvrđena i kod Shirmer I, TF i FTBUT testa (89,8%, 84,5%, 78,0%). Najbolju korelaciju sa citološkim nalazom konjunktivnog skrejpinga imali su RB i FTBUT ($r = 0,707$, $p < 0,001$; $r = -0.507$, $p < 0,001$). **Zaključak.** FTBUT iako često korišćen u kombinaciji sa drugim testovima, samostalno je pokazao značajno visoku senzitivnost i specifičnost, kao i dobru korelaciji sa inflamacijom u sklopu bolesti suvog oka citološki detektovanoj skrejpingom konjunktive.

**Ključne reči:**
**oko, suvo, sindromi; konjunktiva; citologija; osetljivost i specifičnost; dijagnostički testovi; rutinski; dijagnoza, diferencijalna.**

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## Introduction

While investigation on complex mechanism of dry eye disease (DED) has still been ongoing, hiperosmolarity and inflammation that are underlined since the first Dry Eye Workshop (DEWS) report [1] as well as by the OCEAN group [2] are something that all of us are focused on. Advance in the diagnostic tools, but also in therapy are based on these two crucial steps in that vicious circle [3, 4]. New and improved definition of DED, published within the Tear Film and Ocular Surface Society (TFOS) DEWS II Definition and Classification Report [5], emphasizes that hiperosmolarity and inflammation, together with the initial tear film instability and neurosensory abnormalities play etiological roles.

Measuring osmolarity, especially after the introduction of portable *in situ* osmometer (TearLab™, OcuSence, TearLab Corp, San Diego, CA, USA) into clinical practice, seems to be a good way to recognize DED in its early stage [2, 6]. However, there is a question of overlapping of normal subjects and mild form of dry eye (DE) [2]. The rapid point-of-care diagnostic, 9-level test (InflammaDry; Rapid Pathogen Screening, Inc, Sarasota, FL) to detect elevated matrix metalloproteinase, was reported by Sambursky et al. [7] as a diagnostic tool with high sensitivity and specificity when detecting DE related inflammation. Messmer et al. [8] identified the presence of ocular surface inflammation in 40% of confirmed DE patients with this diagnostic test. Although the time-consuming laboratory test, the conjunctival scraping was introduced by Versura et al. [9] as a reliable method to diagnose and score ocular surface inflammation in DE.

We were interested in exploring relation of some commonly used clinical tests available to ophthalmologists in our country with DED- related inflammation measured by conjunctival scraping cytology, in order to make the decision easier as to start an anti-inflammatory treatment.

## Methods

We examined 100 subjects (200 eyes), 88 woman and 12 men. Mean age ± standard deviaton (SD) was 50.17 ± 16.74 years. Eighty of them were referred to us by rheumatologists and general practitioners either during evaluation for the Sjögren syndrome (SS – 30 patients), or because of dry eye symptoms (50 patients). The control group was made of 20 patients in evaluation for cataract surgery, with no DE related symptoms. The exclusion criteria were any ocular surgery that was performed in the period of one year, contact lens wear, topical eye therapy (if only tear substitutes, they had to be suspended at least 8 hours before the examination), entropion, ectropion, or other lid closure problems as well as ocular allergies, or presence of anterior blepharitis. The patients suspected to have the SS were not yet under any kind of systemic anti-inflammatory therapy. The study was approved by the Ethics Committee, of the Faculty of Medicine, Belgrade University. All patients signed an informed consent form.

We performed the following clinical tests: Schirmer without anesthesia (Schirmer I), fluorescein tear break up time (FTBUT), Rose Bengal (RB), Lid Parallel Conjuctival Folds (LIPCOF), Tear Meniscus Height (TMH) and Tear Ferning (TF). Eyelids were inspected for meibomian gland dysfunction (MGD). We also performed scraping of tarsal conjunctiva in order to evaluate ocular surface inflammation. Symptoms were evaluated on the basis of the Ocular Surface Disease Index (OSDI) and McMonnies questionnaires.

To confirm the DE diagnosis in our study, we considered results from a group of three clinical tests. These three tests, the Schirmer I, FTBUT and RB, represented the ophthalmological part of testing for SS according to the Copenhagen criteria, but proved useful in diagnosing DE out of SS context, also [10]. Eighty patients, as we expected, had the dry eye disease, since one, or both eyes were positive in 2 of 3 clinical tests. Twenty patients from this symptomatic group had some form of MGD. In the control group, no eye met these criteria. One patient from the control group had MGD, without the signs, or symptoms of DED. Bearing in mind that we analyzed separately both eyes, we found that 139 eyes were positive and 61 negative for DED. We also graded the DE severity from 0 to 4 according to the DEWS report score system [1]. Numbers of eyes within different grades are presented in Table 1.

**Table 1**
**Distribution of eyes according to dry eye severity with the 0–4 score system from the DEWS report**

| Dry eye severity | Number of eyes | % | Cumulative % |
|---|---|---|---|
| 0 | 37 | 18.5 | 18.5 |
| 1 | 54 | 27.0 | 45.5 |
| 2 | 75 | 37.5 | 83.0 |
| 3 | 23 | 11.5 | 94.5 |
| 4 | 11 | 5.5 | 100.0 |
| Total | 200 | 100.0 | |

**DEWS – Dry Eye Workshop Severity.**

All tests were performed during one examination in the morning by two examiners. First, we examined the patients' TMH and LIPCOF. TMH was measured by slit-lamp. We registered the values as 0.3 mm, 0.2 mm, 0.1 mm, less than 0.1 mm, using the slit –lamp microscope with objective lens graticule in 0.1 units. For the LIPCOF test, we registered in the temporal zone the values as no folds, ½ of fold in the temporal zone, one fold less that 0.2 mm height, two folds 0.2 mm height, 3 folds or more over 0.2 mm. Although similar, these stages, are not completely analogous to those most commonly used, described by Höh et al.[11] Instead of using a term normal meniscus tear height, we used the value of 0.2 mm as a cut-off value between the stages. Other authors also used this value as a normal one [12], and considered pathological if below [13]. We also divided the Stage 1 by Hö h into two stages with present folds, in order to form four grades as the DEWS dry eye severity score system has. Then we performed the Schirmer I, FTBUT and RB test as outlined in the DEWS report [14]. After folding the Schirmer paper strip at the notch, we placed the shorter part under the temporal one-third of the lower lid of both eyes. The patients were asked to close their eyes. We measured the length of wetting from the

notch after 5 minutes, and the cut-off value was ≤ 10 mm/5 min. For FTBUT we applied sodium fluorescein with the impregnated strips and used the average value of three times measured the elapsed time from blink till appearance of the first break in the tear film. The cut-off value was ≤ 10 mm. Punctate staining of the ocular surface, after applying topical anesthesia and Rose Bengal dye was graded with the van Bijsterveld system, with the cut-off value ≥ 4. The TF test was performed by collecting the tear sample from the inferior tear meniscus by using an Eppendof automatic micropipette with a single use 1–10 μL Eppendof Tips. The collected tear sample was pipetted onto a clean microscope slide and allowed to air-dry for 10 minutes. Ferning of the tear was observed by phase contrast light microscope at the magnification level of ×20 and ×40 and quantified according to the Rolando grading scale [15]. Scraping of both upper and lower conjunctiva was performed with a hockey knife at the end of the clinical (slit lamp) examination, in topical anesthesia. The samples were air-dried at room temperature, fixed in methanol and then stained with May-Grunwald–Giemsa. We counted the number of neutrophils, lymphocytes and monocytes under the phase contrast light microscopes in 50 microscopic fields at ×40 as described by Versura et al. [9], and graded inflammation by the Conjuctival Scraping Cytology Scoring System.

We compared each clinical test with the scraping scores and calculated the sensitivity, specificity, positive and negative predictive value (PPV and NPV). To determine the relationship between all the tests we used the Pearson's correlation coefficient r, since all of the tests were parametric. The results of both questionnaires were compared with the conjunctival scrapings of worse eye and we made the comparison between the different age groups (younger, or equal to 60 versus older than 60).

The data were statistically evaluated by using the SPSS version 20 (IBM Corp. Released 2011, the SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp).

## Results

The average value of scraping scores for the group of eyes diagnosed as dry according to the Copenhagen criteria was 5.33 ± 1.99 (95% CI 5.00–5.66), while the average value for the group of non-dry eyes was 2.75 ± 2.04 (95% CI 2.23–3.28). The difference in the average scraping score between the two groups was found to be highly statistically significant by the Student's *t*-test ($t = 8.368$; $p < 0.001$).

The average scraping scores for the different groups of eyes were graded according to the DEWS report and presented in Figure 1. Most overlapping occurs between normal eyes and eyes with a mild form of a dry eye. The difference between all other DEWS groups was statistically significant ($F = 43.197$; $p < 0.001$).

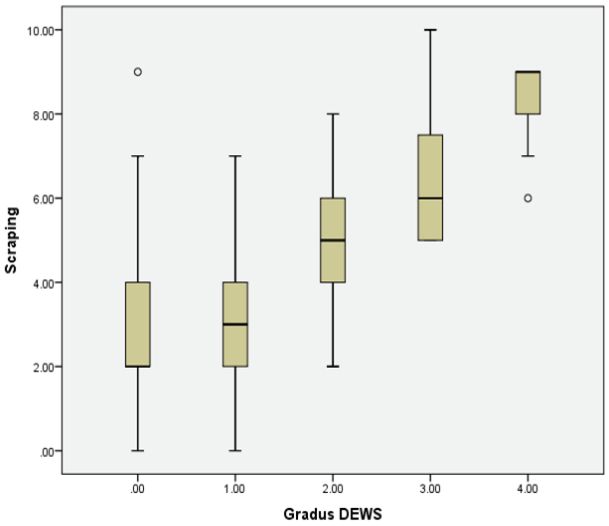


**Fig. 1 – The mean scraping scores in different dry eye severity groups.**
**The average scraping scores for the different groups of eyes graded as in the DEWS report show that the most of the overlapping we have between normal eyes and the ones that have a mild form of dry eye. The difference among all the other DEWS groups is statistically significant ($F = 43.197$, $p < 0.001$).**
**DEWS – Dry Eye Workshop Severity.**

Of all clinical tests that we used, as compared to the conjunctival scraping, the FTBUT as a single test had the best sensitivity (93.6%). The LIPCOF and TMH also had a high sensitivity (92.2% and 80.9%, respectively). The highest specificity was found with RB (93.2%), but it was also high with Schirmer I, TF and FTBUT (89.8%, 84.5%, 78.0%, respectively) (Table 2).

All the tests were in a statistically significant correlation with the conjunctival scraping and among themselves. RB and FTBUT had the highest correlation factor with conjunctival scraping ($r = 0.707$, $p < 0.001$; $r = -0.507$, $p < 0.001$). Among the clinical tests, the best correlation was found between FTBUT and RB ($r = -0.620$, $p < 0.001$), and FTBUT and TF ($r = -0.535$, $p < 0.001$) (Table 3).

**Table 2**
**Sensitivity (Se), specificity (Sp), PPV and NPV of clinical tests compared to the conjunctival scraping cytology**

| Parameters | FTBUT | RB | Sch I | LIPCOF | TMH | TF |
|---|---|---|---|---|---|---|
| Se (%) | 93.6 | 45.4 | 41.1 | 92.2 | 80.9 | 59.9 |
| Sp (%) | 77.9 | 93.2 | 89.8 | 33.9 | 44.1 | 84.5 |
| PPV | 0.91 | 0.94 | 0.91 | 0.77 | 0.78 | 0.89 |
| NPV | 0.85 | 0.42 | 0.39 | 0.65 | 0.49 | 0.48 |

**PPV – positive predictive value; NPV – negative predictive value; FTBUT – Fluorescein Tear Break Up Time; RB – Rose Bengal; Sch I – Schirmer I; LIPCOF – Lid Parallel Conjunctival Folds; TMH – Tear Meniscus Height; TF – Tear Ferning.**

**Table 3**

**Correlation of all tests**

| Test | | FTBUT | Scraping | Sch I | RB | TF | TMH | LIPCOF |
|---|---|---|---|---|---|---|---|---|
| FTBUT | r | 1 | -0.507** | 0.504** | -0.620** | -0.535** | 0.422** | -0.292** |
| | p | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | n | 200 | 200 | 200 | 200 | 190 | 200 | 200 |
| Scraping | r | -0.507** | 1 | -0.383** | 0.707** | 0.486** | -0.352** | 0.328** |
| | p | 0.000 | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | n | 200 | 200 | 200 | 200 | 190 | 200 | 200 |
| Sch I | r | 0.504** | -0.383** | 1 | -0.373** | -0.342** | 0.237** | -0.233** |
| | p | 0.000 | 0.000 | | 0.000 | 0.000 | 0.001 | 0.001 |
| | n | 200 | 200 | 200 | 200 | 190 | 200 | 200 |
| RB | r | -0.620** | 0.707** | -0.373** | 1 | 0.433** | -0.380** | 0.300** |
| | p | 0.000 | 0.000 | 0.000 | | 0.000 | 0.000 | 0.000 |
| | n | 200 | 200 | 200 | 200 | 190 | 200 | 200 |
| TF | r | -0.535** | 0.486** | -0.342** | 0.433** | 1 | -0.385** | 0.318** |
| | p | 0.000 | 0.000 | 0.000 | 0.000 | | 0.000 | 0.000 |
| | n | 190 | 190 | 189 | 190 | 190 | 190 | 190 |
| TMH | r | 0.422** | -0.352** | 0.237** | -0.380** | -0.385** | 1 | -0.377** |
| | p | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | | 0.000 |
| | n | 200 | 200 | 200 | 200 | 190 | 200 | 200 |
| LIPCOF | r | -0.292** | 0.328** | -0.233** | 0.300** | 0.318** | -0.377** | 1 |
| | p | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | |
| | n | 200 | 200 | 200 | 200 | 190 | 200 | 200 |

**FTBUT – Fluorescein Tear Break Up Time; Sch I – Schirmer I; RB – Rose Bengal; TF – Tear Ferning; TMH – Tear Meniscus Height; LIPCOF – Lid Parallel Conjunctival Folds; r - Pearson's correlation coefficient; **$p$ – significant at level < 0.01; n – number of eyes.**

We analyzed the results of McMonnies and OSDI questionnaires and they were in a positive correlation (r = 0.644; $p < 0.001$). When we compared them with the inflammatory cell scores acquired with scraping of tarsal conjunctiva of worse eye, we found that the correlation coefficient was r = 0.315 for the McMonnies questionnaire which was highly significant ($p = 0.001$), and correlation with the OSDI questionnaire was significantly positive as well (r = 0.290; $p = 0.003$). The patients with a higher score of inflammatory cells in tarsal conjunctiva had a higher score on both questionnaires.

When comparing the results of conjunctival scraping of patients younger than 60, we found a positive correlation on the questionnaires that was highly significant (McMonnies r = 0.349; $p = 0.002$, OSDI r = 0.341; $p = 0.003$). For the patients over 60 years of age, we found no correlation between the scraping results and neither of two questionnaires (McMonnies r = 0.011; $p = 0.956$, OSDI r = 0.221; $p = 0.278$). The correlation between the results of two questionnaires in both age group was positive (Group ≤ 60 r = 0.684; $p < 0.001$, Group > 60 r = 0.619; $p = 0.001$).

The average score of conjuctival scraping was higher in the group over 60 years of age than in the group of subjects aged under 60 years, and the difference was statistically significant (t = -1.991, $p = 0.049$). For the over 60 years of age group, the average scraping score was 5.77, and for the under 60 years of age group, it was 4.74.

There was no difference between two age groups in the average scores of both questionnaires (McMonnies $t = 0.927$; $p = 0.356$, OSDI $t = -1.495$; $p = 0.138$).

Of all examined patients, 88% of them were women (88 out of 100 patients). In the group diagnosed with DED, 91.2% were women, which was statistically higher ($\chi^2 = 4.001$; $p = 0.045$) than in the control group (75%).

## Discussion

In the vicious circle of DED, the inflammation is something that comes after tear film instability and hyperosmolarity, [1,2,16] which might explain why, in our study, the conjuctival scraping could not show the clear distinction between the normal and mild dry eyes. Other authors state that in moderately severe dry eye, there is an (often subclinical) inflammatory reaction of the ocular surface and the lacrimal gland [17, 18], and so was confirmed in our study. That suggests that an anti-inflammatory treatment is needed in all except mild stage. Still, confirmation of presence of inflammation should make our decision easier to add this treatment to already existing artificial tears.

The relation between the inflammation and some of clinical symptoms and signs of DED was suggested before [19]. The diagnostic value of the clinical DE tests has been evaluated many times so far [2, 20]. We were interested in their correlation with the DE-related inflammation.

FTBUT compared to the conjunctival scraping as a measure of DE-related inflammation in our study showed the best balance between sensitivity (93.6%) and specificity (77.9%). There was a strong correlation between the FTBUT and conjunctival scraping as well as with the RB and TF.

Alves et al. [20] also reported that the FTBUT sensitivity was 72.3% while specificity was 100%, and they correlated the best with other clinical tests they applied in diagnosing the dry eye in different diseases.

Versura et al. [6] found a strong correlation between FTBUT and tear osmolarity, although in their study, this correlation did not increase in its strength as dry eye severity did.

Discrepancy between the symptoms and signs is a reason why we cannot rely on questionnaires only when it comes to the DE diagnosis and staging of disease [21]. The new definition of DED, published within the DEWS II report, addresses this problem of discrepancy between the signs and symptoms in some patients through the recognition of role for the neurophysiology in the sensory aspect of the disease [22]. In our study, a poor correlation was evident in the group of patients over 60 years of age, where the average scraping score was higher. In their study, Vehof et al. [23] found that the increased age was a predictor of fewer symptoms than signs. This should make us more careful when ruling out, or staging DED in the older population.

## Conclusion

FTBUT, though often used in many combinations of the DED tests, showed in our study a remarkably high sensitivity and specificity on its own, when correlated with the DED-related inflammation. RB and FTBUT had the highest correlation factor with the conjunctival scraping. A poor correlation was found between the symptoms and DE-related inflammation in the patients over 60 years of age. We share the opinion that it is the overall clinical judgment of a clinician that should still be the final judge of DE diagnosis and treatment, but we also believe that it is helpful to have a harder scientific evidence to guide our decision on an anti-inflammatory therapy inclusion in DE treatment.

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Received on November 9, 2017.
Accepted on December 1, 2017.
Online First December, 2017.

ORIGINAL ARTICLE

UDC: 616.314-08
https://doi.org/10.2298/VSP170925183P

# Efficacy of several rotary systems in removal of two different obturation materials during endodontic retreatment

## Efikasnost različitih rotirajućih sistema u uklanjanju dva opturaciona materijala pri endodontskom retretmanu

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**Abstract**

**Background/Aim.** In order to achieve good results in endodontic retreatment, satisfactory removal of filling material and adequate debridement of the root canal is necessary. The aim of this *in vitro* study was to evaluate the efficacy of three rotary systems in removing gutta-percha/AH Plus and RealSeal SE obturation materials during retreatment using scanning electron microscopy. **Methods.** A total of 72 freshly extracted mandibular first incisors were enlarged to a size #30 using iRaCe NiTi instruments. Teeth were randomly divided into 6 groups of 12 specimens each. 36 teeth (groups 1, 2 and 3) were filled with AH Plus®/gutta-percha and another 36 (groups 4, 5 and 6) with Resilon (RealSeal SE system), both using lateral condensation technique. In groups 1 and 4, the retreatment was performed using the ProFile System, in groups 2 and 5 using the ProTaper Universal Retreatment System and in groups 3 and 6 using the D-RaCe system. After retreatment the teeth were split vertically into halves and efficacy of retreatment techniques was evaluated by scanning electron microscopy. The assessment and comparisons of 3 parameters: smear layer, filling debris and surface profile irregularities were made using a predefined scale. These 3 parameters were evaluated in the coronal, middle and apical thirds of the root. Statistical analysis was performed using the Kruskal-Wallis test with the Bonferroni post-hoc test. **Results.** In the AH Plus/gutta-percha samples filling debris removal was significantly better when the D-RaCe and ProTaper System were used compared to the ProFile in the apical third. Less dentin irregularities were observed when the ProTaper was used compared to the ProFile system ($p = 0.0139$). In the RealSeal samples, no significant differences were found between the retreatment methods. **Conclusion.** None of the instrumentation technique completely removed filling material from the root canal, which implies the need for more research in this field. The apical third of the root canal was the most complicated area in terms of complete smear layer and filling debris removal and presence of surface profile irregularities regardless the filling materials.

**Key words:**
**gutta-percha; microscopy, electron, scanning; root canal filling materials; root canal therapy; treatment outcome.**

**Apstrakt**

**Uvod/Cilj.** U cilju postizanja dobrih rezultata u endodontskom retretmanu, neophodno je omogućiti zadovoljavajuće uklanjanje opturacionog materijala i adekvatan debridman kanala korena zuba. Cilj ove *in vitro* studije bio je evaluacija efikasnosti tri sistema rotirajućih instrumenata u uklanjanju gutaperke/AH Plus silera i RealSeal SE sistema tokom retretmana, primenom skenirajuće elektronske mikroskopije. **Metode.** Kanali korenova 72 sveže ekstrahirana donja centralna inciziva su preparisani primenom rotirajućih iRaCe NiTi instrumenata do veličine #30. Zubi su nasumično podeljeni u 6 grupa od po 12 uzoraka. Ukupno 36 zuba (grupe 1, 2 i 3) opturisani su gutaperkom sa AH Plus silerom, a preostalih 36 zuba (grupe 4, 5 i 6) RealSeal SE sistemom, primenom tehnike hladne lateralne kondenzacije. U grupama 1 i 4 retretman je obavljen primenom ProFile Sistema, u grupama 2 i 5 primenom ProTaper Universal Retreatment sistema, a u grupama 3 i 6 primenom D-RaCe Sistema. Posle retretmana zubi su presečeni longitudinalno na polovine a efikasnost metoda retretmana ocenjivana je pomoću skenirajuće elektronske mikroskopije. Upoređivanje tri parametra (prisustvo razmaznog sloja, debris od ostataka opturacionog materiajala i iregularnost površine) obavljeno je pomoću prethodno definisane skale vrednosti. Ova tri parametra su ocenjivana u koronarnoj, srednjoj i apikalnoj trećini korena zuba. Statistička analiza je obavljena primenom Kruskal-Wallis testa sa Bonferroni *post-hoc* testom. **Rezultati.** U uzorcima opturisanim AH Plus/gutaperkom uklanjanje debrisa bilo je statistički značajno bolje primenom D-RaCe i ProTaper sistema u odnosu na ProFile sistem u apikalnoj trećini ($p < 0.05$). Utvrđeno je manje iregularnosti površine dentina kada je korišćen ProTaper system u poređenju sa

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ProFile sistemom ($p = 0.0139$). Kod uzoraka opturisanih RealSeal SE sistemom nisu utvrđene statistički značajne razlike između ispitivanih metoda retretmana. **Zaključak.** Ni jedna od tehnika instrumentacije nije omogućila kompletno uklanjanje opturacionog materijala iz kanala korena zuba. Sve ispitivane tehnike retretmana su bile manje efikasne u apikalnoj trećini kanala korena, nezavisno od vrste opturacionog materijala.

**Ključne reči:**
**gutaperka; mikroskopija, elektronska, skenirajuća; zub, materijali za punjenje korenskog kanala; lečenje, ishod.**

## Introduction

The main goal of nonsurgical root canal retreatment is to reestablish healthy periapical tissues following ineffective root canal treatment, or reinfection [1]. Removal of as much filling material as possible from the inadequately prepared and filled root canal systems would appear to be essential to uncover remaining necrotic tissue or bacteria that may be responsible for periapical inflammation and persistent disease [2]. Thus, in order to achieve good results, it is necessary to perform the adequate debridement of the root canal after the satisfactory removal of previous filling material.

Gutta-percha (GP) is certainly the most commonly used filling material in endodontics. Resilon (Pentron Corp., Wallingford, CT, USA) was introduced relatively recently as a synthetic polymer-based alternative to GP [3]. A polycaprolactone thermoplastic material with bioactive glass, bismuth, and barium salts as fillers has handling properties similar to GP. This material induces a chemical interaction that leads to the formation of a single resin block, which adheres to the root canal walls [4].

When taken into account that Resilon has similar sealing ability as GP with the AH Plus sealer [5], it could be expected that this material can be removed in similar ways as GP. Beside numerous studies concerning its physical, chemical and biological properties, removal of Resilon from root canal has also been investigated [1, 4, 6–8].

There are many different techniques for removal of root canal filling material: solvents [9, 10], hand, rotary [6, 8, 11, 12] and ultrasonic instruments [13, 14], heat-carrying instruments, laser [15, 16] , or a combination of these techniques [17]. The rotary nickel–titanium (NiTi) systems are preferred in endodontic retreatment because of their safety, efficiency and speed [18–21]. In order to improve endodontic retreatment procedure, especially designed the NiTi rotary instruments were developed. In this study, the instruments especially developed for retreatment were used, such as the D-RaCe System (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and ProTaper Universal Retreatment System (PTUS) (Dentsply, Maillefer, Ballaigues, Switzerland) and also, ProFile rotary system (Dentsply Maillefer, Ballaigues, Switzerland) as a conventional system.

Scanning electron microscopy (SEM) was proved to be the adequate method of evaluation of dentin walls after root canal retreatment [1, 4, 14, 19, 22]. According to Pirani et al. [14], SEM observation is the only technique available to observe the smear layer and organic and filling debris in a retreated root canal.

The aim of this study was to evaluate the effectiveness of 3 different rotary instruments (ProFile, PTUS and D-RaCe System) in removing GP/AH Plus, or Resilon filling material from the previously *in vitro* filled root canals using SEM.

## Methods

This study was conducted *in vitro* on 72 freshly extracted human lower first incisors, extracted for orthodontic reasons, or due to periodontal disease. The teeth with immature apices, the presence of external resorption, or any root damage were excluded from the experiment, as well as teeth with two canals or calcifications.

The soft tissue and calculus were removed mechanically from the root surfaces. Two longitudinal grooves of 1-mm depth were prepared with a diamond bur on the lingual and labialsurfaces of each root to facilitate vertical splitting for the SEM analysis after retreatment. After preparation of access cavity, working length was determined by a size 10 K-file (FKG, La Chaux-de-Fonds, Switzerland) 1 mm shorter than its appearance at the apical foramen.

An initial endodontic treatment was performed using the iRaCe rotary NiTi instruments (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland), R1 15/.06, R2 25/.04 and R3 30/.04, using a crown-down sequence, according to the manufacturer recommendations. The irrigation protocol, maintaining patency of the root canals and use of the rotary engine motor were as described in study by Pešić et al. [23].

Before obturation, the samples were randomly divided into 6 groups of 12 specimens each. Thirty-six teeth (groups 1, 2 and 3) were filled with the GP cones (VDW, Munich, Germany) and the AH Plus® sealer (Dentsply DeTrey, Konstanz, Germany), and another 36 (groups 4, 5 and 6) with the Resilon filling material, RealSeal SE system (SybronEndo, Orange, CA, USA), both using the lateral condensation technique. The coronal surface of Resilon groups was light cured for 40 s.

A high-speed hand-piece with water cooling was used for cutting the crowns of the teeth, 14 mm apically from the working lenght to equalize the volume of the filling material in the samples as much as possible. The roots were sealed with GC Fuji II (GC Corporation, Tokyo, Japan). The quality of root canal filling was assessed using the digital radiographs taken in two different directions. The samples were stored for 21 days at 37 C and 100% humidity in an incubator (INCUCELL, MMM Group, Germany) to allow the complete setting of the sealer, as described by Pešić et al. [23]. For the purpose of objectivity, the initial treatment and retreatment procedures were performed by a single operator.

Three different rotary systems were used for retreatment: ProFile rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) in groups 1 and 4; ProTaper Universal Retreatment System (PTUS) (Dentsply Maillefer, Ballaigues,

Switzerland) in groups 2 and 5 and D-RaCe rotary system (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) in groups 3 and 6.

The protocol for each tested retreatment techniques, including the irrigation protocol and rotary engine motor handling procedure, were as described in Pešić et al. [23] study. Apical enlargement during retreatment was up to size 40 for all of these three techniques. The parameters for completion the retreatment were: no more filling material visible on the instruments, or root canal walls and smoothness of root canal walls.

*Scanning electron microscopy evaluation method*

After retreatment, the samples were sectioned in half using a chisel. Random halves were dehydrated in graded alcohol concentrations, dried, and then gold-sputtered (BALTEC, SCD 005 SPUTTER COATER) and observed by SEM (JEOL JSM 6460 LV with EDS device Oxford Instruments INCA; JEOL, Tokyo, Japan).

After a general survey of the root canal walls, the SEM photos of each third of the root canal were taken: at magnification of ×1000 to score the smear layer and inorganic debris at the coronal, middle, and apical thirds (Figure 1), and at ×200 to evaluate the surface profile. The images were saved digitally and individually scored blind by 2 trained operators.

In the selected SEM pictures, the absence, or presence of smear layer and filling debris was rated and scored using a predefined scale [24, 25] by an independent observer. The dentin surface profile was assessed by evaluating the presence of grooves, pits, and predentin areas, also using the predefined scale (Table 1). Each root canal was divided into 3 portions (coronal, middle, apical), and each portion was evaluated independently.

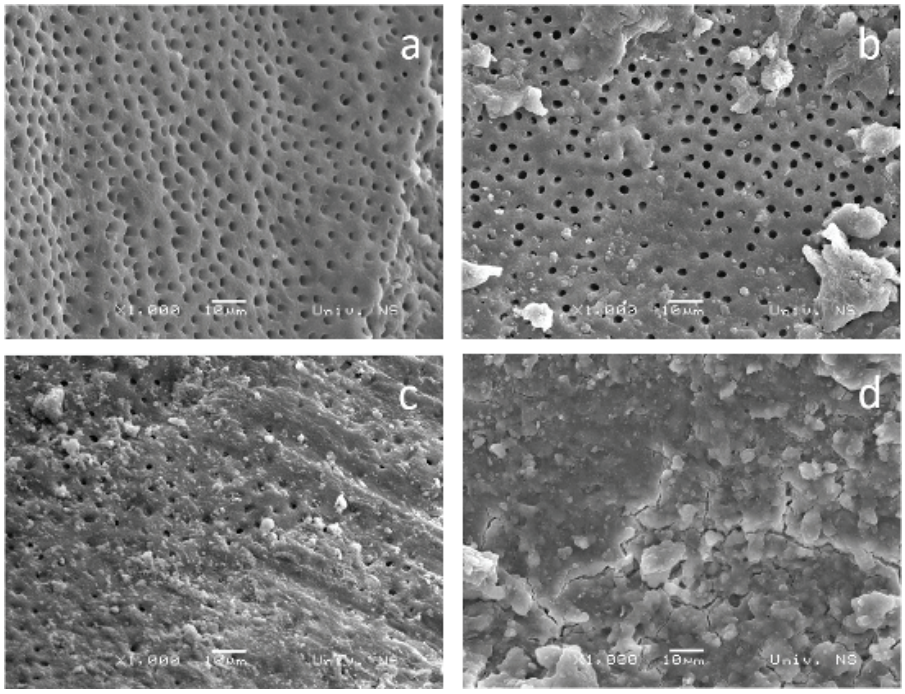


**Fig. 1 – Representative scanning electron microscopy micrographs of samples from the root middle third showing presence of filling debris: a) absent filling debris, b) minimal presence of debris with less than 25% of the area, c) debris often present, d) debris present everywhere and covering dentin surface (Original magnification ×1000).**

**Table 1**

**Scale of values assigned to 3 different parameters evaluated**

| Parameter | Scale of values | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Smear layer | Smear layer absent, more than 75% of tubules exposed and free from smear layer | Present in limited areas, less than 75% of tubules uncovere; tubules partially opened | Present, tubules visible in limited areas and partially closed; less than 50% of dentinal tubules visible | Homogeneous smear layer present above all dentin, dentinal tubules not visible. |
| Filling debris | Absent | Minimal presence (less than 25% of the area) | Often present | Present everywhere and covering dentin surface |
| Surface profile | Absence of irregularities | Isolated irregularities and grooves | Partially irregular, with limited non-instrumented areas | Irregular with grooves, areas of non-instrumented dentin |

*Statistical analysis*

The Kruskal-Wallis test was used to evaluate differences between the mean values of smear layer, filling debris and surface profile of the 3 retreatment methods in the 3 different root canal portions. The level of significance was set at $p < 0.05$. Also, the pairwise comparison tests (Kruskal-Wallis) were done for each pair of retreatment methods and each pair of root canal portions, and significances were adjusted with the Bonferroni correction for multiple comparisons; a level of significance was established at $p < 0.05$.

## Results

*Smear layer*

In the AH Plus/GP groups, the smear layer was observed in several portions of dentin root walls in all the retreatment techniques. The statistical differences ($p = 0.0454$) were found between the 3 retreatment methods only in the coronal third (Table 2). The following pairwise comparison tests, adjusted with the Bonferroni corrections, showed no significant differences between the retreatment methods (Table 2).

In the RealSeal groups, similar amounts of smear layer were observed in the groups 4, 5 and 6. No significant differences ($p > 0.05$) were found between the 3 retreatment methods (Table 2).

In all groups, the smear layer islands were found especially in the apical thirds. When comparing the presence of smear layer in the coronal, middle and apical thirds, the significant differences were found in almost every retreatment technique (Table 2), regardless of the material that was removed from the root canal. The apical third was the one with the most smear layer and the coronal one with the least.

*Filling debris*

In the AH Plus/GP samples, the filling debris was observed in all the retreatment groups. The greatest amount of remaining filling material was found in the apical thirds. A statistical difference ($p = 0.0038$) was found between the 3 retreatment methods only in the apical thirds (Table 2). The ProFile instruments were the least efficient in removing the obturation material. The following pairwise comparison tests adjusted with the Bonferroni corrections showed a significant difference between D-RaCe and ProFile instruments, and between PTUS and ProFile (Table 2).

**Table 2**

**Efficacy of three different retreatment techniques in removing of two obturation materials in each third of the root**

| Characteristics/filling waterial | Retreatment methods | | | *p* |
|---|---|---|---|---|
| | ProFile | PTUS | D-RaCe | |
| Smear layer (mm), mean ± SD | | | | |
| AH Plus/gutta-percha | | | | |
| coronal | $1.58 \pm 0.90^{a,1}$ | $1.08 \pm 0.29^{a,1}$ | $1.00 \pm 0.00^{a,1}$ | 0.0454 |
| middle | $1.92 \pm 1.165^{a,1}$ | $1.33 \pm 0.49^{a,1,2}$ | $1.25 \pm 0.451^{a,1,2}$ | 0.2574 |
| apical | $2.33 \pm 1.37^{a,1}$ | $1.89 \pm 0.83^{a,2}$ | $1.58 \pm 0.67^{a,2}$ | 0.4227 |
| *p* | 0.3301 | 0.0232 | 0.0189 | |
| Real Seal | | | | |
| coronal | $1.00 \pm 0^{a,1}$ | $1.00 \pm 0^{a,1}$ | $1.00 \pm 0^{a,1}$ | 1.00 |
| midlle | $1.33 \pm 0.49^{a,1,2}$ | $1.17 \pm 0.39^{a,1,2}$ | $1.25 \pm 0.45^{a,1,2}$ | 0.6491 |
| apical | $1.67 \pm 0.78^{a,2}$ | $1.58 \pm 0.79^{a,2}$ | $1.50 \pm 0.52^{a,2}$ | 0.9186 |
| *p* | 0.0202 | 0.0327 | 0.0205 | |
| Presence of filling debris, mm, mean ± SD | | | | |
| AH Plus/gutta-percha | | | | |
| coronal | $1.67 \pm 0.78^{1}$ | $1.33 \pm 0.49^{1}$ | $1.17 \pm 0.39$ | 0.1698 |
| midlle | $2.25 \pm 1.05^{1,2}$ | $1.50 \pm 0.67^{1}$ | $1.67 \pm 0.49^{2}$ | 0.1216 |
| apical | $2.75 \pm 0.87^{a,2}$ | $1.75 \pm 0.62^{bc,1}$ | $1.67 \pm 065^{c,1,2}$ | 0.0038 |
| *p* | 0.0271 | 0.2326 | 0.0361 | |
| Real Seal | | | | |
| coronal | $1.08 \pm 0.29^{1}$ | $1.25 \pm 0.45^{1}$ | $1.00 \pm 0.00^{1}$ | 0.1475 |
| midlle | $1.42 \pm 0.51^{1,2}$ | $1.50 \pm 0.67^{1,2}$ | $1.50 \pm 0.52^{2}$ | 0.9267 |
| apical | $2.00 \pm 060^{2}$ | $2.08 \pm 0.79^{2}$ | $1.67 \pm 0.65^{2,3}$ | 0.2995 |
| *p* | 0.0009 | 0.0203 | 0.0065 | |
| Surfice profile, mm, mean ± SD | | | | |
| AH Plus/gutta-percha | | | | |
| coronal | $1.08 \pm 0.29$ | $1.00 \pm 0.00^{1}$ | $1.00 \pm 0.00^{1}$ | 0.3679 |
| midlle | $1.42 \pm 0.51^{a,1}$ | $1.00 \pm 0.00^{b,1,2}$ | $1.17 \pm 0.39^{ab,1,2}$ | 0.0378 |
| apical | $1.67 \pm 0.78^{1}$ | $1.42 \pm 0.51^{3}$ | $1.50 \pm 0.52^{2}$ | 0.7575 |
| *p* | 0.0655 | 0.0035 | 0.0126 | |
| Real Seal | | | | |
| coronal | $1.00 \pm 0.00^{1}$ | $1.08 \pm 0.29^{1}$ | $1.00 \pm 0.00^{1}$ | 0.3679 |
| midlle | $1.08 \pm 0.29^{1,2}$ | $1.08 \pm 0.29^{1}$ | $1.00 \pm 0.00^{1,2}$ | 0.5977 |
| apical | $1.58 \pm 0.51^{3}$ | $1.33 \pm 0.49^{1}$ | $1.42 \pm 0.51^{3}$ | 0.4650 |
| *p* | 0.0012 | 0.1738 | 0.0035 | |

**Horizontally: different superscript letters indicate a significant difference between the groups in each anatomical third of the root; Vertically: different superscript number indicate a significant difference between the thirds in each instrument group. PTUS – proTAper Universal Retreatment System; SD – standard devitaion.**

In the RealSeal groups, the similar amounts of filling debris were observed in the groups 4, 5 and 6. No statistical differences were found between the 3 retreatment methods (Table 2).

When comparing the presence of filling debris in different portions of the root, the apical third was the area with significantly more filling debris ($p < 0.05$) regardless of the method used for removal, except when the PTUS system was used for the removal of GP/AH Plus sealer (Table 2).

*Surface profile*

Concerning the tested instrumentation techniques, in the AH Plus/GP samples, the significant differences in the surface profile appearance were found in the middle thirds (Table 2). The following pairwise comparison tests adjusted with the Bonferroni corrections showed less dentin irregularities when the PTUS system was used compared to the ProFile system ($p = 0.0139$).

The 3 retreatment groups showed similar canal morphology in the samples filled with the RealSeal material, without a significant differences (Table 2).

Comparing the samples filled with the AH Plus/GP and RealSeal for each retreatment method, a significant difference was found only when the ProFile system was used, concerning the smear layer and filling debris evaluation (Table 3). In terms of surface profile irregularities, significant differences were not found. Significantly more smear layer was observed in the AH Plus/GP samples compared to the RealSeal samples in the coronal third (Table 3).

Significantly less amount of filling debris was found in the RealSeal group in the coronal, middle and apical third of the root canal, after retreatment using the ProFile system (Table 3). No significant differences were found when efficacy of other tested instrumentation techniques were compared in two tested filling materials.

**Table 3**
**Comparison between two filling materials (RealSeal vs. AH Plus/gutta-percha) in the ProFile retreatment groups**

| Characteristics | *p* |
|---|---|
| Smear layer | |
| coronal | 0.0325 |
| middle | 0.2385 |
| apical | 0.2688 |
| Filling debris | |
| coronal | 0.0248 |
| middle | 0.0412 |
| apical | 0.0217 |

## Discussion

Removing all root fillings is a prerequisite of nonsurgical retreatment in order to uncover the remnants of necrotic tissue, or bacteria that might have caused the previous failure of the treatment [26]. Therefore, one of the expected root canal filling material good properties is to be easily removable.

In this study, the SEM evaluation was used because it allows the observation of smear layer morphology, presence of debris inside dentinal tubules and root canal orifices and morphology of intertubular dentin [27]. According to Pirani et al. [14], all other possible techniques (including microcomputed tomography) are insufficient to detect these features. Although the SEM evaluation may seem to have no clinical significance, it gives opportunity to detect and compare efficacy of different instruments in endodontic retreatment. The results of this study showed that all of the instrumentation techniques left filling residue inside the root canals, which is in accordance with other studies [4, 7, 8, 14, 22, 28].

The use of rotary instrumentation in removing the root canal filling material is expected to be more efficient compared to hand files. Also, the rotary instrumentation is proved to be safer compared to hand instruments concerning the amount of apically extruded debris [23], which certainly may be the cause of endodontic failure. In the present study, the PTUS and D-RaCe systems, which have been specially developed for retreatment, were used, and their efficacy was evaluated and compared to each other and to the ProFile System, which is commonly used in an initial endodontic treatment as well as in retreatment.

Comparing efficiancy of each instrumentation technique in removing two different materials, significant difference was found only when the ProFile system was used. No significant differences regarding the removal of RealSeal system, compared to AH Plus/GP were found when other tested techniques were used. This result indicates that techniques used for GP removal can also be applied to the Resilon-filled teeth.

In some studies that used SEM as a method of evaluation, the amount of remaining filling material was less in the teeth obturated with Resilon comparing to the GP/sealer [1, 4, 7]. Other studies showed that differences in the amount of remaining filling material were not statistically significant regarding to different filling methods [28]. It is questionable, however, whether all these studies are comparable with this one, because of different retreatment methods used in these investigations.

In this study, the SEM evaluation showed remnants of the filling material in all 3 analyzed root thirds, which is in accordance with other studies [4, 8, 10]. This investigation showed that the absence of filling materials on the instruments and smoothness of root canal walls was not a valid criteria to demonstrate complete removal of the filling material from the canal walls, as explained by Zarei et al. [29].

In the samples obturated with AH Plus and GP there was no significant differences between the tested instruments in smear layer removal. In terms of filling debris, the removal instruments specially designed for retreatment were more efficient than ProFile in the apical thirds of the roots. This result can be very important clinically since the microorganisms remained in the apical portion of the root canal have been considered to be the main cause of the endodontic treatment failure [30]. The fact that the PTUS and D-RaCe systems were more efficient than ProFile in the apical third of the root canal in terms of GP removal indicates that the espe-

cially designed instruments should be used in the retreatment cases. Also, specific design characteristics of the instruments may affect their efficiency during retreatment [31, 32]. The results of these studies may be related to the convex triangular cross-section of the PTUS and D-Race instruments that renders their internal mass larger than the internal mass of the ProFile instruments.

In this study, additional instruments were used during retreatment, which was proven to result in a statistically significant improvement in the root canal wall cleanliness [31]. In a Marques et al. [31] study, there was no significant difference when D-RaCe and PTUS with use of additional instruments were compared which is similar to this investigation.

In the samples obturated with the RealSeal system, all retreatment techniques showed similar performances in terms of the smear layer morphology, amount of debris and surface profile. It is in compliance with results of other studies [7, 8, 28].

The apical third of the root was the area with the greatest amount of smear layer, filling residual debris and surface profile irregularities, with grooves and zones of non-instrumented dentin regardless the filling materials, which was in accordance with other studies [7, 22]. As previous studies concluded [14, 33], the apical instrumentation with a no. 40 instrument is probably insufficient for the complete removal of the filling debris plugs present in all dentinal tubules, which was also a result of this investigation.

## Conclusion

The SEM evaluation proved to be very efficient method for observing the root canal walls morphology after endodontic retreatment. None of the instrumentation technique completely removed filling material from the root canal, which implies the need for more research in this field. The apical third of the root canal was the most complicated area in terms of complete smear layer and filling debris removal and presence of surface profile irregularities regardless the filling materials. Further research should be directed towards finding solutions for better apical debridement. In the apical thirds, the instruments especially developed for retreatment were significantly more efficient in removal of AH Plus/GP than the ProFile instruments, which should be considered when performing endodontic retreatment.

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Received on September 25, 2017.
Revised on December 4, 2017.
Accepted on December 11, 2017.
Online First December, 2017.

ORIGINAL ARTICLE


UDC: 617.55:616.351-006-089-036.8
https://doi.org/10.2298/VSP171020187A

# Influence of individual surgeon volume on early postoperative outcomes after rectal cancer resection

## Uticaj individualnog hirurškog volumena na rane postoperativne ishode posle resekcije rektuma zbog karcinoma

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**Abstract**

**Background/Aim.** Surgeon-specific experience as measured by procedure volume can have a significant impact on survival of patients with rectal cancer (RC). The aim of this study was to determine whether an individual surgeon-specific volume of procedure influences early postoperative outcomes as well as to determine the strength of different groups of annual surgeon volume (ASV), as a predictor of outcomes in patients after RC resection up to 30 days postoperatively. **Methods.** This retrospective observational single center study involved a cohort of 546 patients of both sexes, operated for a 10-year period due to RC. Patients were divided into three groups, according to the annual volume of RC procedures of a surgeon who operated them. Seven outcomes were analyzed: the incidence of colorectal anastomotic dehiscence (CRAD), operative time, intraoperative blood loss, hospital stay, in-hospital death, the status of the circumferential resection margin (CRM) and the total mesorectal excision (TME) with number of lymph nodes, as well as some risk factors (several independent, dependent and "confusing" variables) of importance for the outcome, to explain the difference. The strength of each group of surgeons and their effect on early outcome of treatment were determined. **Results.** The majority of surgeons (77.7%) belonged to the low and medium ASV, which performed a slightly higher number of surgeries (281) than the high volume group. The high-volume surgeon group was associated with significantly better results in four outcomes (CRAD, operating time, CRM, TME and number of lymph nodes). **Conclusion.** In our surgical institution, the high volume surgeon remains an important predictor of success of the RC surgery.

**Key words:**
**hemorrhage; length of stay; surgeons; surgical procedures, operative; surgical wound dehiscence; rectal neoplasms; survival; treatment outcome.**

**Apstrakt**

**Uvod/Cilj.** Individualno hirurško iskustvo mereno brojem procedura može značajno uticati na preživljavanje bolesnika sa karcinomom rektuma (KR). Cilj ovog rada bio je da se utvrdi da li individualni volumen procedure hirurga utiče na rane postoperativne ishode i da li jačina različitih grupa procedura hirurškog volumena, kao predskazatelja ishoda kod bolesnika sa KR do 30 dana posle hirurškog lečenja. **Metode.** Sprovedena je retrospektivna jednocentrična studija sa kohortom od 546 bolesnika oba pola, elektivno operisanih u 10-godišnjem period zbog KR. Bolesnici su bili podeljeni u tri grupe, shodno godišnjem volumenu procedura 18 ordinirajućih hirurga u kolorektalnoj hirurgiji. Analizirano je sedam ishoda: stopa dehiscencije kolorektalne anastomoze (DKRA), vreme trajanja operacije, intraoperativni gubitak

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krvi, dužina hospitalizacije, intrahospitalna smrtnost, patohistološki status cirkumferencijalne resekcione margine (CRM) i totalna mezorektalna ekscizija (TME) sa brojem limfnih nodusa, kao i drugi faktori rizika (više nezavisnih, zavisnih i "zbunjujućih" varijabli) od značaja za ishod operativnog lečenja i objašnjenje razlike. Utvrđivana je jačina svake grupe hirurga i njihov uticaj na rane ishode lečenja. **Rezultati.** Većina hirurga (77,7%) pripadala je grupi sa niskim i srednjim volumenom procedura koja je izvela nešto veći broj operacija (281). Grupa hirurga sa visokim volumenom imala je značajno bolje rezultate u četiri ishoda (stopa DKRA, vreme trajanja operacije, status CRM I TME sa brojem limfnih nodusa). **Zaključak.** U našoj hirurškoj ustanovi, visoki hirurški volumen je važan predskazatelj uspešnog ishoda u hirurškom lečenju karcinoma rektuma.

**Ključne reči:**
**krvarenje; hospitalizacija, dužina; hirurzi; hirurgija, operativne procedure; rana, hirurška, dehiscencija; rektum, neoplazme; preživljavanje; lečenje, ishod.**

## Introduction

Since the anterior resection has become the method of choice for treatment of rectal cancer (RC), the effect of anastomotic dehiscence (AD) on postoperative morbidity, mortality and the cost of treatment is the growing concern among surgeons [1–3]. The colorectal AD (CRAD) is the most common and the most severe complication for all reconstructions and represents the "Achilles heel" of each surgeon [4, 5]. The frequency of CRAD varies in patients' series of different authors, ranging between 3% and 19%, after elective surgery, and in operations with total mesorectal excision (TME) it is usually higher, 10–24%. The rate of postoperative mortality due to CRAD is between 12% and 27%, which is the cause of death in up to one third of the deaths after the RC surgery [5–10]. In order to heal anastomosis, in addition to systemic and local factors, operating technique (technical factors) plays a crucial role. Operating technique varies from school to school and from surgeon to surgeon in fulfilling the basic conditions of anastomosis [10, 11]. Many studies [12–15] examined risk factors for CRAD, but there is no consensus on the role of each of them. The development of CRAD remains unpredictable in many patients [16]. Some authors identify intraoperative blood loss of 200 mL or more and operative time of 200 minutes or longer as factors the increased risk of postoperative AD [16]. The quality of surgical resection plays a critical role in the outcome of patients with colon and RC. Adequate surgical resection is important for regional cancer control [17]. A negative (R0) circumferential resection margin (CRM) is described as one of the most important factors that decrease the rate of local recurrence in RC [18]. The rate of CRM positivity is widely used as a quality indicator in RC care [19] and serves as a useful indicator of the quality of surgery [20]. The American College of Surgeons and the American Society of Clinical Oncology endorsed a minimum 12-lymph node count as a quality measure for better outcome in colon cancer patients [17]. It is important to adhere to strict oncologic principles for cancer resections, including high vascular ligation and complete 'en bloc' resection of the mesocolon, lymphadenectomy and CRM (for RC) [17].

Numerous studies have examined the association between the surgeon case volume and clinical outcome for various procedures and have shown higher surgeon volume to be associated with better outcomes [10]. Surgeon-specific experience as measured by procedure volume can have a significant impact on survival in patients with RC [21]. The best early postoperative surgical outcomes are achieved in centres where there are high annual volume surgeons attending these patients [22].

Despite the considerable body of research in this area, little is known about the mechanisms underlying the observed associations between the surgical volume and postoperative outcomes in patients with RC [23].

The aim of our retrospective observational study was to show that individual surgeon-specific volume of procedure influences early postoperative outcome and determine the strength of different groups of annual surgical volume (ASV), as a predictor of outcome in patients after RC resection, up to 30 days postoperatively.

## Methods

Retrospective, single center study, with a cohort of 546 patients operated in the period between January 1st, 2007 and December 30th, 2016 at the Clinic for General and Abdominal Surgery of the Clinical Center in Banjaluka the Republic of srpska, Bosnia and Herzegovina.

The trial included patients of both sexes with RC, clinical stages T1 N0, T2 N0-2 and T3 N0-2. The study did not include patients in the stage T4, with a local irresectable process, local recurrence and dissemination of the disease. In all patients the anterior resection of the rectum with an open approach was performed. Colorectal anastomosis (CRA) was hand-sewn in 208 patients (single inverting extramucosal sutures in a single layer) and in 338 patients – a stapler technique (ILP 29–33 mm). Anastomotic technique and the creation of protective ileo- or transverse colostomies (in selective cases) depended exclusively on the individual assessment and the skill of the operating surgeon. Primary chemotherapy and radiotherapy were carried out individually in accordance with the decision of the multidisciplinary team. Clinical parameters of CRAD were: appearance of purulent or fecal content in drainage tube, pelvic abscess, peritonitis, rectovaginal fistula and the appearance of purulent discharge per *recti*. For the detection of eventual AD, a digital rectal examination, anoscopy and/or proctoscopy (for low rectal anastomoses) and radiographic contrast control were used in selective cases (grade "B" of CRAD).

Eighteen surgeons who operated the patients were classified into three groups based on their annual volume of colorectal procedures: low volume of the surgeon (≤ 5 procedures), medium (6–10 procedures) and high volume (> 10 procedures) [10].

Seven outcomes were analyzed: the incidence of CRAD, operative time, intraoperative blood loss, hospital stay, in-hospital death, the status of the CRM and the TME with number of lymph nodes, as well as some risk factors (several independent, dependent and "confusing" variables) of importance for the outcome.

All collected data were analyzed using commercial statistical software SPSS Statistics for Windows version 21. Depending on the results of the Kolmogorov-Smirnov test, the statistical significance between the groups was checked by *t*-test for independent groups, or alternatively by ANOVA. Some variables are presented in the form of frequencies of particular features (categories), and the significance of difference was determined using the $\chi^2$ test or the Mann-Whitney test and the Kruskal-Wallis test. A value of $p < 0.05$ was considered statistically significant.

## Results

During the 10 year study period, 18 surgeons in a single hospital performed 546 resections for RC. The majority of surgeons were in the low and medium volume groups (Table 1). A statistically significant difference was found among the groups.

Patients were mostly male (61.53%). The largest number of RC operations was performed in the period from 2013 to 2015 (60, 73, 62, respectively), and the largest number of patients was in the seventh and eighth decade of life (351). Compared to the age groups of patients, an approximately equal burden of surgeons in all three ASV groups was found ($\chi^2 = 14.76$; $p = 0.255$). Although surgeons from a high volume group more frequently operated patients with the Charlson Comorbidity Index (CCI) ≥ 3, no statistically significant difference was found among surgeon volume groups ($x^2 = 5.723$; $p = 0.214$).

Most of the patients (396 or 72.5%) had the loss of body weight over 15% from the beginning of the disease until the operation. Surgeons from low volume group operated a slightly higher number of patients whose weight loss was registered. Statistical analysis showed no significant difference, but a clear tendency ($p = 0.054$) amongsurgeon volume groups.

Most of the patients belonged to the American Society Anesthesiologists (ASA) risk classification system scores II and III (432 or 79.12%). All three surgeon volume groups operated patients with ASA II ($\chi^2 = 6.286$, $p = 0.347$) in over 50% of cases.

Table 2 shows the distribution of RC to the segments of the rectum. All of the patients with RC in the distal segment were operated by surgeons from high volume group. The highest percentage of low anastomosis (≤ 12 cm from the anal verge) was performed in the group with the highest ASV (210/326). There was a statistically significant correlation between the volume groups.

**Table 1**
**Annual surgical volume (ASV) of 18 surgeons in colorectal resections**

| Parameter | Surgens (n) | Annual surgical procedures | |
|---|---|---|---|
| | | mean ± SD | min-max |
| Surgeon volume group | | | |
| low (≤ 5) | 8 | 4.54 ± 0.27 | 4.20–5.00 |
| medium (6–10) | 6 | 8.83 ± 0.83 | 7.30–9.86 |
| high (> 10) | 4 | 18.33 ± 1.17 | 16.60–19.20 |
| Total | 18 | 9.03 ± 5.50 | 4.20–19.20 |
| Comparison (ANOVA) | | F = 465.8; $p < 0.001$ | |
| Tukey test | | $p < 0.001$ | |

**min – minimum; max – maximum; SD – standard deviation.**

**Table 2**
**Anatomic location of rectal cancer (RC) according to annual surgical volume**

| Rectal segment | ASV | | | Total |
|---|---|---|---|---|
| | ≤ 5 | 6–10 | > 10 | |
| Lower | | | | |
| number | 0 | 0 | 99 | 99 |
| rows (%) | 0.0 | 0.0 | 100.0 | 100.0 |
| columns (%) | 0.0 | 0.0 | 37.4 | 18.1 |
| Middle | | | | |
| number | 48 | 68 | 111 | 227 |
| rows (%) | 21.1 | 30.0 | 48.9 | 100.0 |
| columns (%) | 36.1 | 45.9 | 41.9 | 41.6 |
| Upper | | | | |
| number | 85 | 80 | 55 | 220 |
| rows (%) | 38.6 | 36.3 | 25.0 | 100.0 |
| columns (%) | 63.9 | 54.0 | 20.7 | 40.3 |
| Total | | | | |
| number | 133 | 148 | 265 | 546 |
| rows (%) | 24.4 | 27.1 | 48.5 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2 = 21.85$; $p < 0.001$ | | | |

The majority of patients had an T3 N0-2 stage of RC (69%, 377/546). There was no significant association between the TNM tumor stage and ASV ($\chi^2 = 8.58$, $p = 0.072$).

All three of the surgeon volume groups relative to the type of newly discovered abdomen conditions during the operation did not differ significantly. Higher number of newly discovered states (n = 83) in the high volume group correlates with a large number of performed surgeries ($\chi 2 = 5.24$, $p = 0.983$).

After anterior resection of the rectum, CRA was created by manual sewing in 208 patients and in 338 with a stapler technique. Statistically significantly was lesser use of hand-sewn technique by surgeons within the low annual volume group. In patients with CRA stapling technique hospitalization lasted up to 8 days in 61.5% of cases ($p < 0.001$). There was no statistically significant difference between techniques of anastomosis and mortally outcome (hand-sewn 4.3%, stapler 5.0%). Regardless of the age of the surgeon, the application of stapler technique was dominant.

There was a statistically significant correlation between the type of mesorectal excision and localization of RC. TME is applied for carcinomas in the distal third and for carcinomas in the middle third in a significant number of patients (Table 3).

There was no statistically significant correlation between the type of mesorectal excision and CRAD ($\chi^2 = 0.48$; $p = 0.48$), as well between the type of mesorectal excision and in-hospital mortality ($\chi^2 = 0.55$; $p = 0.457$).

A statistically significantly lower number of tissue stapler rings with defect was registered in the high volume group of surgeons (Table 4).

**Table 3**

**Relation between rectal cancer and type of mesorectal excision (ME)**

| Rectal segment | Type of ME | | Total |
|---|---|---|---|
| | partial | total | |
| Lower | | | |
| number | 0 | 99 | 99 |
| rows (%) | 0.0 | 100.0 | 100.0 |
| columns (%) | 0.0 | 41.6 | 20.8 |
| Middle | | | |
| number | 95 | 132 | 227 |
| rows (%) | 41.9 | 58.1 | 100.0 |
| columns (%) | 30.8 | 55.5 | 43.1 |
| Upper | | | |
| number | 213 | 7 | 220 |
| rows (%) | 96.8 | 3.2 | 100.0 |
| columns (%) | 69.2 | 2.9 | 36.1 |
| Total | | | |
| number | 308 | 238 | 546 |
| rows (%) | 56.4 | 43.6 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2 = 293.77$; $p < 0.001$ | | |

**Table 4**

**Relation between annual surgical volume (ASV) and the status of tissue stapler rings**

| ASV (number of operations) | Stapler rings | | Total |
|---|---|---|---|
| | complete | with defect | |
| 0 ≤5 | | | |
| number | 90 | 12 | 102 |
| rows (%) | 88.2 | 11.8 | 100.0 |
| columns (%) | 28.7 | 48.0 | 38.3 |
| 6–10 | | | |
| number | 85 | 8 | 93 |
| rows (%) | 91.4 | 8.6 | 100.0 |
| columns (%) | 27.2 | 32.0 | 29.6 |
| ≥ 10 | | | |
| number | 138 | 5 | 143 |
| rows (%) | 96.5 | 3.5 | 100.0 |
| columns (%) | 44.1 | 20.0 | 32.1 |
| Total | | | |
| number | 313 | 25 | 338 |
| rows (%) | 92.6 | 7.4 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2 = 6.21$; $p = 0.045$ | | |

The average number of removed lymph nodes in the low volume group was 11 (133 operations), in the medium 13 (148 operations) and in the high volume group 16 (256 operations).

There was no statistically significant difference in the number of patients with intraoperative finding of significant fecal content in the colorectum compared with ASV ($\chi^2$ = 1.359; $p$ = 0.507). A significant amount of fecal content was registered in a total of 20.9% of patients. The majority of patients had fecal trace amounts in the colorectal lumen at the operation (468).

There was no statistically significant difference in percentage of patients with fecal contamination of peritoneum and operative wounds (78) compared to ASV ($\chi^2$ = 2.154; $p$ = 0.341).

The used colon segments for reconstruction were sigma (387), descendens (125) and transversum (34). Relation of ASV ($\chi^2$ = 6.966; $p$ = 0.138) and anastomosis type ($p$ = 1.00) was not statistically significant, but the relation between the type of anastomosis and the used colon segment was statistically significant ($\chi^2$ = 62.414; $p$ < 0.001). A high percentage of using sigma for CRA with stapler technique (72.4%) was detected. Also, no statistically significant difference in the association between the occurrence of CRAD ($\chi^2$ = 2.054; $p$ = 0.374) and lethal outcome ($\chi^2$ = 1.299; $p$ = 0.562) was established.

A statistically significant association between ASV and the decision for the formation of a protective stoma was not established ($\chi^2$ = 1.416; $p$ = 0.852), but there was statistically significant association with the height of anastomosis to 6 cm from the anal edge ($\chi^2$ = 115.77; $p$ < 0.001), when protective ileostomes were used in 70.2% of cases. In the hand-sewn technique of CRA creation, in 87.5% no protective stoma was made, but the stapler technique in 84.0% of cases followed the creation of ileostomy. There was statistically significant association with CRA ($\chi^2$ = 36.927; $p$ < 0.001). Also, the appearance of CRAD in 62.5% of cases was followed by diverting colostoma ($\chi^2$ = 32.837; $p$ < 0.001). Statistically significant association existed with a lethal outcome ($\chi^2$ = 9.953; $p$ = 0.005), in 25% of cases with colostoma.

The highest percentage of colorectal resections was performed by surgeons from a high volume group in a period of less than 3 hours (Table 5). Statistical analysis confirmed the significant difference in this parameter among groups ($\chi^2$ = 6.357; $p$ = 0.042).

There was statistically significantly higher percentage of patients with blood loss during surgery (> 200 mL) in the group of high volume surgeons (Table 6). The CRAD and lethal outcome were statistically significantly related to blood loss ($p$ < 0.001).

**Table 5**
**Distribution of operation time according to the annual surgical volume (ASV)**

| Operation interval | ASV (number of operations) | | | Total |
|---|---|---|---|---|
| | ≤ 5 | 6–10 | > 10 | |
| 1–3 hours | | | | |
| number | 37 | 48 | 106 | 191 |
| rows (%) | 19.4 | 25.1 | 55.5 | 100.0 |
| columns (%) | 27.8 | 32.4 | 40.0 | 35.0 |
| > 3 hours | | | | |
| number | 96 | 100 | 159 | 355 |
| rows (%) | 27.0 | 28.2 | 44.8 | 100.0 |
| columns (%) | 72.2 | 67.6 | 60.0 | 65.0 |
| Total | | | | |
| number | 133 | 148 | 265 | 546 |
| rows (%) | 24.4 | 27.1 | 48.5 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2$ = 6.357; $p$ = 0.042 | | | |

**Table 6**
**Relation of annual surgical volume (ASV) and intraoperative blood loss**

| Surgeon volume groups (per number of operations) | Blood loss (> 200 mL) | | Total |
|---|---|---|---|
| | no | yes | |
| 0 ≤5 | | | |
| number | 94 | 39 | 133 |
| rows (%) | 70.7 | 29.3 | 100.0 |
| columns (%) | 23.9 | 25.5 | 24.4 |
| 6–10 | | | |
| number | 119 | 29 | 148 |
| rows (%) | 80.4 | 19.6 | 100.0 |
| columns (%) | 30.3 | 19.0 | 27.1 |
| ≥ 10 | | | |
| number | 180 | 85 | 265 |
| rows (%) | 67.9 | 32.1 | 100.0 |
| columns (%) | 45.8 | 55.6 | 48.5 |
| Total | | | |
| number | 393 | 153 | 546 |
| rows (%) | 72.0 | 28.0 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2$ = 7.482; $p$ = 0.024 | | |

The CRAD of grade B and C (according to the International Study Group of Rectal Cancer – ISGRC) was verified in 53 (9.7%) of patients. The smallest percentage of CRAD was verified in the high volume surgeon group. Statistical analysis confirmed the significance among the groups ($\chi^2$ = 6.992; $p$ = 0.030) (Table 7).

A statistically significant association between ASV and early/late CRAD was not established, neither was the statistically significant association of early/late CRAD and lethal outcome ($\chi^2$ = 1.657; $p$ = 0.198).

A statistically significantly lower percentage of positive CRMs was determined after a resection procedure by the high volume surgeons (Table 8).

There was a significant difference in the length of hospitalization between the low and medium volume group. There was no significant difference between the medium and the high volume group (Table 9). The longest stay in the Intensive Care Unit (ICU) and in the hospital were registered in patients operated by surgeons from the high volume group. Between a surgeon of the medium and high volume groups, no statistically significant difference was found.

The total mortality rate of up to 30 days was 4.8% (26/546). Although the incidence of the lethal outcome was the smallest in the high volume group (3.8%), there was no statistically significant difference among the groups (Table 10).

There was no statistically significant association between ASV and preoperative radiotherapy (Table 11). However, these patients had a statistically significant increase of CRAD ($p < 0.001$). Out of 42 patients with preoperative radiotherapy, 11 (26.2%) developed CRAD. More frequent deaths in this group (7.1%) were noted, but without a statistically significant difference compared to the group without preoperative radiotherapy.

**Table 7**

**Distribution of colorectal anastomotic dehiscence (CRAD) according to the annual surgical volume (ASV)**

| CRAD | ASV (number of operations) | | | Total |
|---|---|---|---|---|
| | ≤ 5 | 6–10 | > 10 | |
| No | | | | |
| number | 114 | 131 | 248 | 493 |
| rows | 23.1 | 26.6 | 50.3 | 100.0 |
| columns | 85.7 | 88.5 | 93.6 | 90.3 |
| Yes | | | | |
| number | 19 | 17 | 17 | 53 |
| rows (%) | 35.8 | 32.1 | 32.1 | 100.0 |
| columns (%) | 14.3 | 11.5 | 6.4 | 9.7 |
| Total | | | | |
| number | 133 | 148 | 265 | 546 |
| rows (%) | 24.4 | 27.1 | 48.5 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2$ = 6.992; $p$ = 0.030 | | | |

**Table 8**

**Relation of annual surgical volume (ASV) and the status of circumferential resection margin (CRM)**

| ASV (number of operations) | CRM | | Total |
|---|---|---|---|
| | negative (R0) | positive (R1/2) | |
| 0 ≤ 5 | | | |
| number | 114 | 19 | 133 |
| rows (%) | 85.7 | 14.3 | 100.0 |
| columns (%) | 23.1 | 36.5 | 29.8 |
| 6–10 | | | |
| number | 132 | 16 | 148 |
| rows (%) | 89.2 | 10.8 | 100.0 |
| columns (%) | 26.7 | 30.8 | 28.7 |
| ≥ 10 | | | |
| number | 248 | 17 | 265 |
| rows (%) | 93.6 | 6.4 | 100.0 |
| columns (%) | 50.2 | 32.7 | 41.5 |
| Total | | | |
| number | 494 | 52 | 546 |
| rows (%) | 90.5 | 9.5 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2$ = 6.785; $p$ = 0.034 | | |

**Table 9**

**Days in the Intensive Care Unit (ICU) and in the hospital according to the annual surgical volume (ASV)**

| ASV (number of operations) | Days, mean ± SD | |
|---|---|---|
| | ICU | Hospital |
| [1] ≤ 5 | 0.87 ± 0.77 | 8.88 ± 3.23 |
| [2] 6–10 | 1.26 ± 0.97 | 9.70 ± 4.70 |
| [3] > 10 | 1.34 ± 1.49 | 10.35 ± 5.41 |
| Comparison between ASV groups: | | |
| [1] : [2] | z = 3.66 $p < 0.001$ | z = 1.90 $p = 0.057$ |
| [1] : [3] | z = 4.47 $p < 0.001$ | z = 3.81 $p < 0.001$ |
| [2] : [3] | z = 0.55 $p = 0.58$ | z = 1.84 $p = 0.065$ |

**SD – standard deviation.**

**Table 10**

**Distribution of deaths (during 30 postoperative days) according to the annual surgical volume (ASV)**

| Deaths | ASV (number of operations) | | | Total |
|---|---|---|---|---|
| | ≤ 5 | 6–10 | > 10 | |
| No | | | | |
| number | 127 | 138 | 255 | 520 |
| rows (%) | 24.4 | 26.5 | 49.0 | 1000 |
| columns (%) | 95.5 | 93.2 | 96.2 | 95.2 |
| Yes | | | | |
| number | 6 | 10 | 10 | 26 |
| rows (%) | 23.1 | 38.5 | 38.5 | 100.0 |
| columns (%) | 4.5 | 6.8 | 3.8 | 4.8 |
| Total | | | | |
| number | 133 | 148 | 265 | 546 |
| rows (%) | 24.4 | 27.1 | 48.5 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2 = 1.88$; $p = 0.389$ | | | |

**Table 11**

**Relation of annual surgical volume (ASV) according to preoperative radiation therapy**

| ASV (number of operations) | Preoperative radiotherapy | | Total |
|---|---|---|---|
| | no | yes | |
| 0 ≤ 5 | | | |
| number | 127 | 6 | 133 |
| rows (%) | 95.5 | 4.5 | 100.0 |
| columns (%) | 25.2 | 14.3 | 24.4 |
| 6–10 | | | |
| number | 132 | 16 | 148 |
| rows (%) | 89.2 | 10.8 | 100.0 |
| columns (%) | 26.2 | 38.1 | 27.1 |
| ≥ 10 | | | |
| number | 245 | 20 | 265 |
| rows (%) | 92.5 | 7.5 | 100.0 |
| columns (%) | 48.6 | 47.6 | 48.5 |
| Total | | | |
| number | 504 | 42 | 546 |
| rows (%) | 92.3 | 7.7 | 100.0 |
| columns (%) | 100.0 | 100.0 | 100.0 |
| Comparison | $\chi^2 = 3.930$; $p = 0.140$ | | |

**Discussion**

This study examined the early outcomes of surgical treatment for RC in patients with observation period of up to 30 days postoperatively, in order to show the link between ASV and early outcomes in this field of surgery, especially with the appearance of CRAD. The effect of ASV on the outcome of surgical treatment in RC remains uncertain and it is not clear whether the volume of a hospital or a surgeon is an important predictor of the outcome. It is considered that the surgeon's specific experience, measured by ASV, can have a significant effect on the survival of patients with RC [21].

The development of AD remains unpredictable in many patients undergoing RC surgery. In the analysis of our patients, only clinical criteria for determining AD were used. CRAD was registered in 53 (9.7%) of our patients. AD was nonoperatively treated in 34 patients, while others were surgically treated. The smallest percentage of CRAD is verified in the high volume group. A statistically significant association between ASV and early/late CRAD was not established, nor was a statistically significant association of early/late CRAD and lethal outcome.

The total mortality rate up to 30 days in our patient's series was 4.8% (26/546). Although the incidence of lethal outcome was the smallest in the high volume group (3.8%), no statistically significant difference was found among the groups. CRAD was the direct cause of death in 6 (23%) patients.

There was a significant difference in the length of hospitalization between the low and the medium volume group. There was no significant difference between the medium and high volume group. The longest stay in the ICU was registered in patients operated by surgeons from the high volume group. They were patients with significant comorbidities (CCI $\geq$ 3) and over 70 years of age. Between surgeons of the medium and high volume group no statistically significant difference was found. The mean length of stay in the hospital for our patients with AD was approximately 4 times longer than for patients without AD, which is also consistent with the experience of other authors [6–10].

In this field of surgery as well as for all surgical procedures sex, age, constitution, comorbidity and other factors significantly influence the decision of the surgeon for the type of surgery and the postoperative outcome. The age over 70 years (most of the patients in our study – 351/546) is a significant risk factor for AD [24]. This population of patients, with numerous risk factors, has a higher rate of AD, a more severe clinical course and higher morbidity and mortality [4].

Recent studies have confirmed that weight loss of more than 15% over a period of 6 months preoperatively, due to the associated metabolic imbalance, increases the incidence of complications and mortality [25]. Males (most of our patients) with their anatomical characteristics of the pelvis (narrow pelvis) have increased risk for CRAD, but also propensity for good local disease control (local recurrence) and preservation of vegetative nervous structures. The difficult working conditions in deep and narrow pelvis often make it impossible for a surgeon to technically create anastomosis, so that the rate of CRAD in males is higher in comparison to women who naturally have a broad and shallow pelvis [11].

Most of our RC patients who underwent extensive surgical treatment at the same time had one or more comorbidities. In our study, CCI was used to evaluate preoperative comorbidities [6]. Increased comorbidity was present in ASA patients with $\geq$ 3, causing them to have an increased risk for CRAD [4]. Buchs et al. [14] showed that, with each degree of increase in ASA score, there is a 2.5 times increase in frequency of CRAD.

According to tumor localization, the height of anastomosis is a known independent prognostic factor, not only for the appearance of dehiscence, but also for the local recurrence of the disease. With an increase in the distance of anastomosis from the anal edge, the frequency of dehiscence decreases (distal third 14.1%, mean 8.1% and upper 2.6%) [4].

In T3 and T4 disease stages, especially when there is penetration and infiltration of surrounding tissues, there is a higher incidence of anastomosis dehiscence. Because of that patients staged T4 were not included in our study.

The decision on the selection of the reconstruction colon segment is made by the surgeon during surgery [26]. In our series in 387 (70.9%) patients the sigmoid colon was used for CRA creation. After a low resection of the rectum, the creation of termino-terminal anastomosis as one of the reconstructive methods is technically the simplest, but postoperative functional results (emptying frequency, urgency, continence, fragmentation, use of medicaments) are worse than reconstruction with the reservoir [4].

A meta-analysis of 9 randomized, controlled studies, published in 2001 [27], concluded that there was no proven superiority of the stapler technique over hand-sewn, regardless of the level of anastomosis. In our study, younger surgeons, who more quickly and easily accept advanced techniques in surgery, used a stapler technique more often. According to our results, as well as other authors, AD was similar in both groups. This demonstrates that sutures and stapler technique are equally suitable for colorectal anastomosis. Which anastomosis technique will be applied is decided based on individual surgical experience and the personal preference of surgeons [27–30].

Preoperative neoadjuvant chemoradiotherapy is nowadays often a part of the treatment protocol for patients with RC in order to reduce tumor and its stages, and thus prevent local relapse and achieve greater percentage of sphincter-preserving operations. Radiation adversely affects the healing of anastomosis by causing microangiopathy, so the timing of irradiation is critical. Importantly, negative effects of short-term preoperative radiation and chemotherapy on the healing process of CRA were not observed [31].

Mechanical bowel preparation is an integral part of the general preoperative preparation of the patient. The issue of mechanical intestinal cleansing is the topic of controversy: from the point that it is a requisite for the prevention of complications on anastomosis [32–34], to the point that it is of no importance in elective surgery and that the frequency of dehiscence is twice as higher after mechanical cleaning of the bowel as without it (8.1% : 4%) [32–34]. It has been reliably proven that this preparation, for resection of the colon, is not as important as it is desirable for resection of the rectum. However, these controversies do not change the attitude of

most surgeons today that surgery on the empty bowel is more comfortable and easier [4]. Intraoperative contamination of the operative complex and incision wounds with fecal content containing bacterial flora can seriously compromise the outcome of surgery, which imposes the obligation on all members of the surgical team for careful and pedantic work. If the intestinal lumen at the operation is filled with fecal content, it is necessary to empty and lavage, in order to make the primary CRA possible and safe. In patients with ileus the bowel wall is stretched and edematous which, with increased intraluminal pressure due to the presence of fecal masses and degradation gases, may impair the healing of CRA. Also, in such circumstances and whenever possible, the application of the "double stapler" technique reduces the possibility of contamination as manipulated by a closed lumen hose [35].

The height of the CRA relative to the anal edge is a significant independent risk factor for the appearance of dehiscence, the frequency of which increases with the approach of the anus [4]. Vignali et al. [35] reported on a series of 1,014 CRA stapler surgeries, with a total of 2.9% dehiscence, 7.7% below and 1% above 7 cm from the anal edge. In their multivariance analysis, only the height of anastomosis was an independent prognostic factor.

Higher blood loss during surgery and intraoperative blood transfusion have shown adverse effects on the healing of intestinal anastomosis in experimental and clinical trials [4, 6, 36–39]. In our study, surgeons from the high volume group had a statistically significant increase in blood loss during surgery. This could be due to the fact that they predominantly operated older patients, T3 tumors in the distal third of the rectum with distal anastomoses and the patients with larger CCI.

The duration of the operation depends on several factors: surgical technique, intraoperative complications, previous abdominal surgery, experience of a surgeon and an operational team. More studies have shown that the extended time of over 200 minutes causes changes in the activity of inflammatory mediators and, consequently, ischemic and septic complications [37, 40]. In our study, the largest percentage of surgeries were performed by surgeons from the high volume group over a period of less than 3 hours.

The option of creating a diverting stoma is today controversial. Stoma is a temporary solution, in the case of low CRA in males, in patients with significant comorbidity, neoadjuvant chemoradiotherapy, or in the presence of peritonitis, in order to minimize complications. Protective stoma is a procedure with complications (ischemia, prolapse, stenosis) and therefore this option must be objectively justified [4, 37, 40]. In our study, the statistically significant association of ASV and the decision to form a diverting stoma was not established, but statistically significant association with the height of anastomosis was found, up to 6 cm from the anal edge, where the protective ileostomas were applied in 70.2% of cases. In the hand-sewn technique of CRA in 87.5% of the patients no diverting stoma was made, but 84.0% of cases with the stapler technique followed by the creation of an ileostomy. There was a statistically significant relationship with CRA. Also, the appearance of CRAD in 62.5% of cases was followed by performing diverting colostoma. There was statistically significant association between performing colostomawith lethal outcome (25% of the patients with colostomy).

The outcome of surgery for RC has improved substantially during the past two decades because of the introduction of TME [41]. The leakage rate following TME was 2.7–17% and multivariance analysis showed that the risk of leakage was significantly higher in men, in patients undergoing neoadjuvant radiotherapy, and in anastomoses that were ≤ 6 cm from the anal verge. The authors concluded that low anastomoses created after TME should be protected by a diverting stoma [42, 43]. In our study there was no statistically significant correlation between the type of mesorectal excision and CRAD.

The rate of CRMs positivity is widely used as a quality indicator in RC care. The survival in RC has been shown to be very variable between surgeons and institutions. One of the major factors influencing survival is local recurrence, and this in turn is strongly related to inadequate tumor excision, particularly at the CRM. Fortunately, this is one parameter that the operating surgeon has the power to control. The quality of surgery in particular the skill of resection of the mesorectum at the CRM becomes one of the most important aspects of management. Of 586 patients on whom full clinical follow up was obtained 165 (28.2%) had CRM involvement by carcinoma on pathologic examination [19, 20]. A positive CRM was noted in 2,859 (17.2%) of the 16,619 patients in another study [44]. In a study with 192 patients [18] the R1 rate was 3.6%. In our study, after stapler creating CRA, surgeons checked the integrity of the tissue stapler rings. The observed defects were solved by additional stitching of anastomosis, creating a protective stoma for larger defects, or the formation of a new anastomosis.

Numerous hospitals in the world are considering setting minimum standards for colorectal surgery. One meta-analysis [45] including 47 studies with 1,122,303 patients from 9,649 hospitals and 9,649 surgeons showed that there is an influence of surgeon volume on the outcome with large volumes of high volume surgeons favoring better outcomes. The best outcomes occur in the high-volume hospitals with high-volume surgeons, followed by hospitals with low volume and high-volume surgeons. Also, this meta-analysis showed that mortality rates were not the lowest in studies with high annual volumes of hospitals and surgeons. Studies with a volume of 100 operations per year, compared to the lowest group, had a lower reduction in mortality among groups, than a study where a high group had more than 20 operations compared to a low group. Identifying a clear threshold effect, that is, estimating the relationship between volume and improvement in any outcome is difficult. A potential reason for this may be the high number of hospital with multiple surgeons, so each individual volume is low, while fewer hospitals with few surgeons have each single volume high. Therefore, a high volume for individual surgeons in hospitals can also be a surrogate for quality interventions and whether the volume of the hospital can be a surrogate for the volume of a surgeon. A high-volume surgeon is probably an important predictor of outcomes, but there may be other surgeon groups that achieve excellent results [10, 46]. From the outcome

point, it would be desirable that most cases are operated by high-volume surgeons in high-volume hospitals. It is considered that 10 procedures per year are sufficient. In a hospital with at least 70 cases per year, surgeons who make up to 5 operations per year can get the best results. This could be a message of optimism [10].

Profiling the results of individual surgeons can help identify a surgeon with better results in order to improve the outcome of surgical treatment. Our goal was to give our own contribution to the debate about referring patients with RC to surgeons with a higher annual volume of operations for better outcome.

The limitations of this study may be due to the lack of information on some specificities of the surgeon during surgery and the follow-up on patients after 30 days of observation.

**Conclusion**

Development of AD is unpredictable in many patients after surgical treatment of RC. In our surgical institution with a high annual volume of colorectal surgery, most surgeons belonged to groups with low and medium annual volume of procedures in colorectal surgery (77.7%), with statistically significant difference among groups. In our patients series, the high-volume surgeon group was associated with significantly better results in four (CRAD, operating time, CRM, TME and number of lymph nodes), out of seven analyzed early postoperative outcomes. The high-volume surgeon remains an important predictor of success in the surgical treatment of RC.

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Received on October 20, 2017.
Revised on December 11, 2017.
Accepted on December 18, 2017.
Online First December, 2017.

ORIGINAL ARTICLE


UDC: 617.55-053.2:616.346.2-002-07
https://doi.org/10.2298/VSP171218012S

# Neutrophil myeloperoxidase index in pediatric acute appendicitis

## Neutrofilni mijeloperoksidazni indeks u akutnom apendicitisu kod dece

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**Abstract**

**Background/Aim.** Diagnosis of acute appendicitis (AA) remains the most common dilemma of pediatric surgical team. Our aim was to determine whether the neutrophil myeloperoxidase index (MPXI), in combination with other laboratory and clinical parameters, can be useful in diagnosis and follow-up of AA in children. **Methods.** A prospective investigation of MPXI values in 117 consecutive patients, planned for the surgical intervention due to AA, was performed. The patients were stratified into three groups according to the intraoperative finding: the normal/early, uncomplicated and complicated AA. Laboratory analyses were done preoperativly, on the 1st and on the 3rd postoperative days. **Results.** The statistically significant difference of MPXI values between the uncomplicated and complicated appendicitis before surgery and the positive correlations between the MPXI and C-reactive protein, as well as interleukin-6, before surgery were found. Postoperatively, in the group of uncomplicated, as well as complicated AA, a significant decrease of MPXI was recorded. **Conclusion.** The MPXI may be used as an informative biomarker in the follow-up of AA in children. A wide reference range for the MPXI and individual differences in the values of MPXI in the healthy children, generate difficulties for its use for the initial diagnosis of acute appendicitis. Usefulness of MPXI determination decreases with a delayed diagnosis.

**Key words:**
**appendicitis; appendectomy; child; diagnosis, differential; peroxidase; neutrophils; c-reactive protein; interleukin-6.**

**Apstrakt**

**Uvod/Cilj.** Dijagnoza akutnog apendicitisa (AA) i dalje ostaje jedna od najčešćih dilema u radu pedijatrijskog hirurškog tima. Naš cilj je bio da se ispita da li mijeloperoksidazni indeks (MPXI), u kombinaciji sa drugim laboratorijskim i kliničkim parametrima, može biti koristan u dijagnostici AA kod dece. **Metode.** Sprovedeno je prospektivno ispitivanje vrednosti MPXI kod 117 bolesnika, planiranih za hirušku intervenciju zbog AA. Bolesnici su bili podeljeni u tri grupe: normalni/rani, nekomplikovani (flegmonozni) i komplikovani (gangrenozni i/ili perforativni) AA. Laboratorijska ispitivanja vršena su preoperativno, kao i prvog i trećeg postoperativnog dana. **Rezultati.** Utvrđena je statistički značajna razlika u vrednosti MPXI između nekomplikovanog i komplikovanog apendicitisa, preoperativno. Ispitivanjem korelacije MPXI sa drugim laboratorijskim i kliničkim parametrima, utvrđena je korelacija sa C-reaktivnim proteinom i interleukinom 6, preoperativno. Postoperativno, i u grupi nekomplikovanog, kao i komplikovanog AA, zabeležen je značajan pad vrednosti MPXI. **Zaključak.** Širok opseg referentnih vrednosti i individualne razlike u vrednostima MPXI kod zdrave dece, ograničavaju upotrebu MPXI u dijagnostici akutnog apendicitisa kod dece. Odložena dijagnostika AA smanjuje upotrebnu vrednost MPXI.

**Ključne reči:**
**apendicitis; apendektomija; deca; dijagnoza, diferencijalna; peroksidaze; neutrofili; c-reaktivni protein; interleukin-6.**

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## Introduction

Acute appendicitis (AA) is the most frequent emergency and appendectomy is the most frequent operation in the pediatric abdominal surgery [1]. Despite the new diagnostic methods [scoring systems, ultrasound, computed tomography (CT), nuclear magnetic resonance (NMR)], incidence of negative appendectomy as well as the rate of perforated disease, followed by many possible complications of this two misdiagnostic states, remains the same in some countries [2]. Negative appendectomy is the most commonly defined as the absence of inflammation, or pathology in the appendix after surgical intervention done for suspected appendicitis [3]. A certain rate of these negative explorations is accepted as good surgical practice because the devastating impact of perforated appendicitis. Epidemiological data in certain countries suggest that negative appendectomy could appear with incidence of up to 30% of all suspected appendicitis, especially in girls [4].

A diagnosis of AA in children is more challenging than in adults, due to a lack of cooperation and limited clinical history data. A missed or delayed diagnosis of appendicitis increases the possibility of perforation, which has the highest incidence in young children [5], and results in a fivefold increase in a postoperative complication rate [6]. So, distinguishing a complicated (gangrenous and perforated) from an uncomplicated (flegmonous), possibly conservatively treatable appendicitis, is of a great importance in clinical practice.

Among other laboratory parameters, some of which being used in diagnosing the AA in children, we routinely get the neutrophil myeloperoxidase index (MPXI), which is often unrecognized in clinical practice for this purpose. The MPXI represents quantity of myeloperoxidase (MPO) in the neutrophil population relative to the archetype (normal) population, and is calculated by the hematological autoanalyzer Advia 120/2120 (Siemens), as a standard output during a process of the white blood cell (WBC) differentials.

The objectives of this prospective trial were to investigate the possible relevance of MPXI in diagnosis and follow-up of AA in children as well as to investigate a possible correlation of MPXI trends with the recorded clinical parameters in the AA groups defined according to the intraoperative finding.

## Methods

The patients, 3 to 16 years old, admitted to the Mother and Child Healthcare Institute of Serbia "Dr. Vukan Čupić"and referred for surgery after establishing the diagnosis of AA, were included into the prospective evaluation. Children younger than 3 years were not recruited. The patients with other acute diseases and the patients with intraoperative finding of other abdominal inflammation were excluded from the study. The study was approved by the Ethics Committee of the Mother and Child Healthcare Institute of Serbia (ref. number 8/8, from 08. Apr 2015), and run in line with the Good Clinical Practice and Declaration of Helsinki.

The baseline evaluation included medical history, duration of symptoms and blood sampling. The blood sampling evaluated the complete blood counts (CBC) and C-reactive protein (CRP). The serum obtained by the peripheral venous blood centrifugation was stored at -70 ºC for the later measurements of cytokines. The Pediatric Appendicitis Score (PAS) was calculated at the baseline for every patient (scoring from 1 to 10 as the following: migration of pain-1, anorexia-1, nausea/emesis-1, tenderness in right lower quadrant-2, cough/percussion and hop tenderness-2, pyrexia-1, leukocytosis-1 and polymorphonuclear neutrophilia-1). Two additional blood samplings were performed at the 1st and the 3rd postoperative day for the same laboratory analysis.

The WBC differentials were measured using the hematological autoanalyzer Advia 120/2120 (Siemens AG, Eschborn, Germany). The measurement of MPO activity represented by the MPXI is displayed as an integral output/result. The test uses 4-chloro-1-naphthol as a substrate for the MPO in the granulocytes and black precipitates are formed in these cells. The neutrophils, monocytes and eosinophils are positive while lymphocytes and basophils are the MPO negative cells. The neutrophils are discriminated from the monocytes and eosinophils by the cell optical characteristics and peroxidase content. The MPXI is calculated as MPXI = [(Mean Neutrophil MPO Staining – Expected Staining Index) / Expected Staining Index] × 100, where the Mean Neutrophil MPO staining is the result of the absorbance measurement in the neutrophils. The Expected Staining Index is the expected MPO measurement result for an ideal standard neutrophil population which is maintained by the regular daily calibration [7]. The MPXI changes were analyzed before operation and during the postoperative period. The MPXI values were tested depending on the study groups and the time period that passed between the onset of symptoms and surgical intervention. Also, these values were correlated with the PAS, CRP and interleukin-6 (IL-6), and followed up over three postoperative days.

The determination of the cytokine concentrations in the sera of AA patients was performed on the Beckman Coulter FC500 cytometer using a commercial flow cytometric kit Human Inflammation 20 plex BMS 819, according to the manufacturer's instructions. Due to its role in a direct stimulation of CRP production – a reliable biomarker for cytokine-mediated response in the AA, the values of IL-6 were taken for a statistical analysis, while the values of other tested cytokines were beyond the scope of this article.

For the statistical analysis, we used the GraphPad Prism 5.01 (GraphPad Prism Software Inc. California, USA). Correlations (Spearman rho) and comparisons (Mann–Whitney *U*-test) were calculated for the comparative statistics (z-score and two-tailed *p*). Normality was assessed by using the Kolmogorov-Smirnov test.

## Results

During the period May-October 2015, 117 consecutive patients were included in this prospective analysis. A total of 117 patients were stratified into three groups according to the intraoperative finding. The first group represented the patients with a normal appendix and an early stage of appendicitis (NEAA, n =

21), where normal appendix or mildly swollen (catarrhal appendicitis) was found; the second group consisted of patients with the phlegmonous or uncomplicated appendicitis (UAA, n = 45); the third group were the patients with gangrenous and/or perforated appendicitis noticed as complicated appendicitis (CAA, n = 51). There were 72 male and 45 female patients from 3 to 16 years of age (in average 10.28 ± 4.07 years). The baseline results and results obtained during analysis on the 1st and 3rd postoperative day are shown in Table 1.

*The neutrophil myeloperoxidase index values are the lowest in children with uncomplicated acute appendicitis*

Regarding the differences of MPXI values between the groups at the different time points from surgical intervention, the significantly lower MPXI values before surgery in the UAA group compared to the children in the CAA group were found [-2.83 ± 6.07 vs. -1.01 ± 5.73; $p = 0.0058$, Figure 1A)]. There were no statistically significant differences in the MPXI values between the UAA and NEAA groups nor between the NEAA and CAA groups in the samples taken before surgery.

On the 1st postoperative day, the lowest MPXI values were also in the UAA group with a very high significant difference in comparison with the NEAA group [-4.487 ± 7.125 vs. 2.405 ± 3.000; $p < 0.0001$ (Figure 1B), and a significantly lower one in comparison with the CAA group [-4.487 ± 7.125 vs. -2.10 ± 7.407; $p = 0.0472$ (Figure 1B)]. A statistically significant difference was found between the NEAA and CAA group on the 1st day after surgery as well [2.405 ± 3.000 vs. -2.10 ± 7.407; $p = 0.0101$ (Figure 1B)].

On the 3rd postoperative day, again, we found the lowest MPXI values in the UAA group with a very high significant difference in comparison with the NEAA group [-6.353 ± 8.329 vs 0.400 ± 4.677; $p = 0.0006$ (Figure 1C)], and a significantly lower one in comparison with the CAA group-[(6.353 ± 8.329 vs. -3.444 ± 7.125; $p = 0.0321$ (Figure 2C). There were no statistically significant differences in the MPXI values between the NEAA and CAA groups in the samples taken on the 3rd postoperative day.

*The value of neutrophyl myeloperoxidase index determination changes with delayed diagnosis of acute appendicitis*

In 65 out of 117 patients, the surgery was performed within 24 hours from onset of symptoms and in 52 of them after this period of time. A classification of patients regarding the time duration that elapsed between the onset of symptoms and surgical intervention, revealed the marked differences in comparison to the MPXI values among the NEAA, UAA and CAA groups.

In the patients with the symptoms duration less than 24 hours, in the samples taken before surgery, the lowest MPXI values in the UAA group were found, which were highly significantly lower compared to the NEAA group (-2.7 ± 6.7 vs. 4.8 ± 0.8; $p = 0.0040$) and significantly lower compared to the CAA group [-2.7 ± 6.7 vs. -0.6 ± 6.7; $p = 0.0449$ (Figure 2A)]. The MPXI values of the NEAA group were significantly higher in comparison with the CAA group [4.8 ± 0.8 vs. -0.6 ± 6.7; $p = 0.0224$ (Figure 2A)]. On the contrary, in the patients with symptoms duration more than 24 hours, there was no statistically significant differences in the MPXI values before surgery when the NEAA, UAA and CAA groups were compared (Figure 2A).

On the 1st postoperative day, in the patients with symptoms duration less than 24 hours, the significantly lower MPXI values in the UAA group compared to the NEAA group (-5.4 ± 7.9 vs. 3.5 ± 3.6; $p = 0.0018$) and in comparison with the CAA group was found [-5.4 ± 7.9 vs. -1.8 ± 7.9; $p = 0.0129$ (Figure 2B)]. In the patients with symptoms duration more than 24 hours, the statistically significant differences between the MPXI values emerged as well, and we found the lowest values in the CAA group, significantly lower compared to the NEAA group [-2.5 ± 7.0 vs. 1.9 ± 2.7; $p = 0.0382$ (Figures 2B)]. The MPXI values in the UAA group of patients with the symptoms duration more than 24 hours were lower in comparison to the NEAA group with a high statistical significance [-1.6 ± 2.2 vs. 1.9 ± 2.7; $p = 0.0076$ (Figure 2B)].

On the 3rd postoperative day, in the patients with symptoms duration less than 24 hours, the lowest MPXI values were again in the UAA group, with a very high statistical significance in comparison to the NEAA group [-6.2 ± 6.6 vs. 3.8 ± 3.4; $p = 0.0008$ (Figure 2C)] as well as in comparison to the CAA group [-6.2 ± 6.6 vs. -2.3 ± 7.2; $p = 0.0008$ (Figure 2C)]. In the patients with the symptoms duration more than 24 hours, on the 3rd postoperative day, there was no statistically significant differences in the MPXI values between the NEAA, UAA and CAA groups (Figure 2C).

*The neutrophil myeloperoxidase index do not correlate with the Pediatric Appendicitis Score before surgery*

A significant correlation between the MPXI and PAS values before surgery was not found, neither with some single parameters of this score nor with the absolute number of leukocytes and the percentage of neutrophils.

*The neutrophil myeloperoxidase index correlate positively with C-reactive protein*

In the peripheral blood samples of the total number of patients (n = 117), taken before surgery, a significant positive correlation between the MPXI values and the CRP concentrations was found [Spearman's $r = 0.3014$; $p = 0.0082$ (Figure 3A)]. In the peripheral blood samples taken on the 1st postoperative day, no statistical significance was shown. On the 3rd postoperative day, in the total number of patients, a significant correlation between MPXI and CRP was present again [Spearman's $r = 0.2132$; $p = 0.0370$ (Figure 3B)].

By classifying the patients into three groups, a positive correlation between the MPXI and CRP before surgery was found only in the NEAA group of patients [Spearman's $r = 0.4667$; I = 0.0440 (Figure 3C)]. In the peripheral blood samples taken on the 1st and the 3rd postoperative days in the NEAA, as well as in all samples from the UAA and CAA groups, a statistical significance could not be reached.

**Table 1**

**Parameters in the groups of patients with acute appendicitis on the different time points from surgery**

| Group of patients | Sampling time | PSD (days) mean ± SD | PAS mean ± SD | MPXI mean ± SD | WBC ($10^9$/L) | Ne (%) | Ly (%) | NLR | CRP (mg/L) mean ± SD |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | mean ± SD | | | | |
| NEAA (n = 21) | before surgery | 3.41 ± 2.47 | 6.71 ± 2.05 | -0.20 ± 5.58 | 12.49 ± 4.42 | 69.05 ± 10.45 | 20.42 ± 9.29 | 4.88 ± 4.18 | 48.91 ± 49.52 |
| | 1st postop. day | | | 2.41 ± 3.00 | 11.55 ± 2.90 | 77.31 ± 7.86 | 14.62 ± 6.32 | 6.76 ± 4.67 | 57.80 ± 42.97 |
| | 3rd postop. day | | | 0.40 ± 4.68 | 7.726 ± 2.11 | 60.03 ± 5.36 | 27.38 ± 5.71 | 2.33 ± 0.72 | 38.78 ± 33.37 |
| UAA (n = 45) | before surgery | 1.22 ± 0.67 | 6.89 ± 1.76 | -2.83 ± 6.07 | 16.76 ± 5.61 | 80.27 ± 11.05 | 12.23 ± 8.96 | 10.83 ± 7.74 | 26.28 ± 33.89 |
| | 1st postop. day | | | -4.49 ± 7.12 | 11.88 ± 3.42 | 76.86 ± 7.65 | 15.31 ± 5.98 | 6.20 ± 3.55 | 66.99 ± 56.62 |
| | 3rd postop. day | | | -6.35 ± 8.32 | 7.79 ± 2.36 | 59.08 ± 10.98 | 28.06 ± 10.01 | 2.55 ± 1.45 | 37.01 ± 33.63 |
| CAA (n =51) | before surgery | 1.83 ± 1.31 | 8.02 ± 1.61 | -1.01 ± 5.73 | 19.08 ± 6.99 | 83.10 ± 6.75 | 8.61 ± 4.26 | 14.20 ± 12.45 | 94.36 ± 74.40 |
| | 1st postop. day | | | -2.10 ± 5.84 | 13.36 ± 9.50 | 78.39 ± 7.57 | 13.21 ± 6.24 | 8.49 ± 89.95 | 137.10 ± 7.40 |
| | 3rd postop. day | | | -3.44 ± 7.12 | 10.42 ± 6.75 | 66.93 ± 12.67 | 21.42 ± 10.51 | 4.43 ± 3.33 | 86.25 ± 61.06 |

**PSD – preoperative symptoms duration; PAS – Pediatric Appendicitis Score; MPXI – myeloperoxidase index; WBC – white blood cell; Ne – neutrophils; Ly – lymphocytes; NLR – neutrophil/lymphocyte ratio; CRP – C-reactive protein; NEAA – normal/early acute appendicitis; UAA – uncomplicated acute appendicitis; CAA – complicated acute appendicitis; SD – standard deviation.**

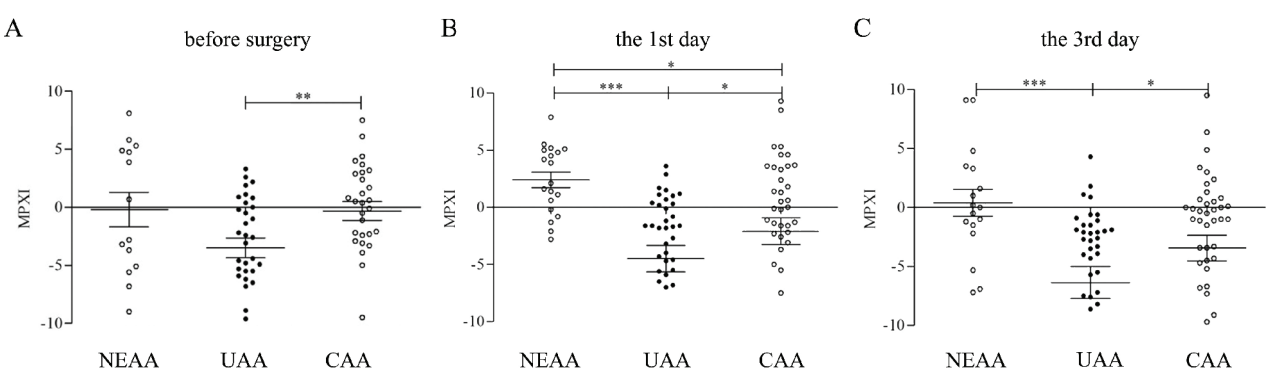


**Fig. 1 – A) Comparison of MPXI among three groups of acute appendicitis (AA) patients before surgery, showing the significantly lower values in the UAA group when compared with the CAA group; B) Comparison of MPXI on the 1st postoperative day showing the lowest values in the UAA group when compared with the NEAA and CAA groups. The MPXI values of CAA group are significantly lower in comparison with the NEAA group; C) Comparison of MP XI on 3rd postoperative day showing the statistically lower values in the UAA group when compared with the NEAA and CAA groups.**
**The Mann-Whitney test (unpaired, two tailed) * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.0001$.**
**For abbreviations see under Table 1.**

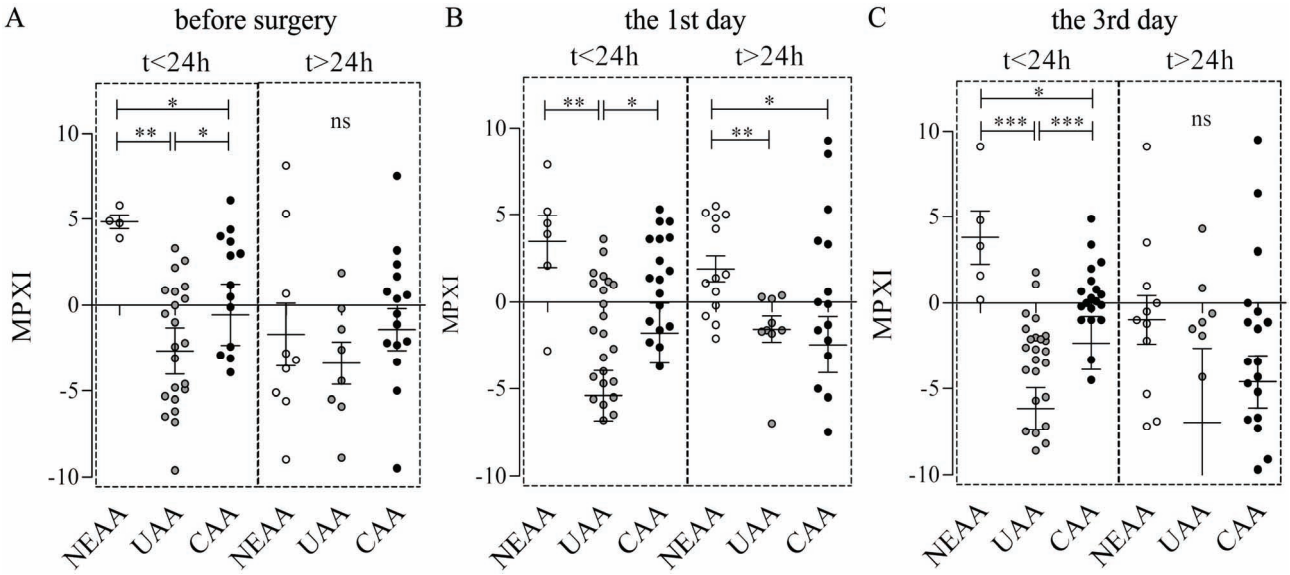


**Fig. 2 – A) Comparison of MPXI values among the NEAA, UAA and CAA groups before surgery, showing the statistically significant differences between all tested groups in the patients who undergone surgery within 24 hours of symptoms' onset (t < 24h), with the lowest values in the UAA group. On the contrary, in the patients with surgical intervention after more than 24 hours of symptoms' onset (t > 24), the differences were insignificant before surgery; B) On the first postoperative day, among the patients who undergone surgery within 24 hours of symptoms' onset, the MPXI values are lowest in the UAA group, with a statistical significance in comparison with the NEAA and the CAA groups. In the group of patients who undergone surgery after more than 24 hours, the MPXI values are highest in the NEAA group, a significantly higher in comparison to the UAA and the CAA groups, with the lowest values in the CAA group; C) On the third postoperative day, in the patients who undergone surgery within 24 hours of symptoms' onset, the MPXI values in the UAA group are lower in comparison with the NEAA and CAA groups with a very high statistical significance. As in measurements before surgery, the MPXI values of NEAA group are significantly higher in comparison to the CAA group on the third postoperative day among the patients with surgery within 24 hours of symptoms' onset. On the contrary, in the patients which undergone surgery after more than 24 hours, the statistically significant differences between all tested groups are lost on the third postoperative day.**
**The Mann-Whitney test (unpaired, two-tailed): * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.0001$; ns – not significant.**
**For abbreviations see under Table 1.**

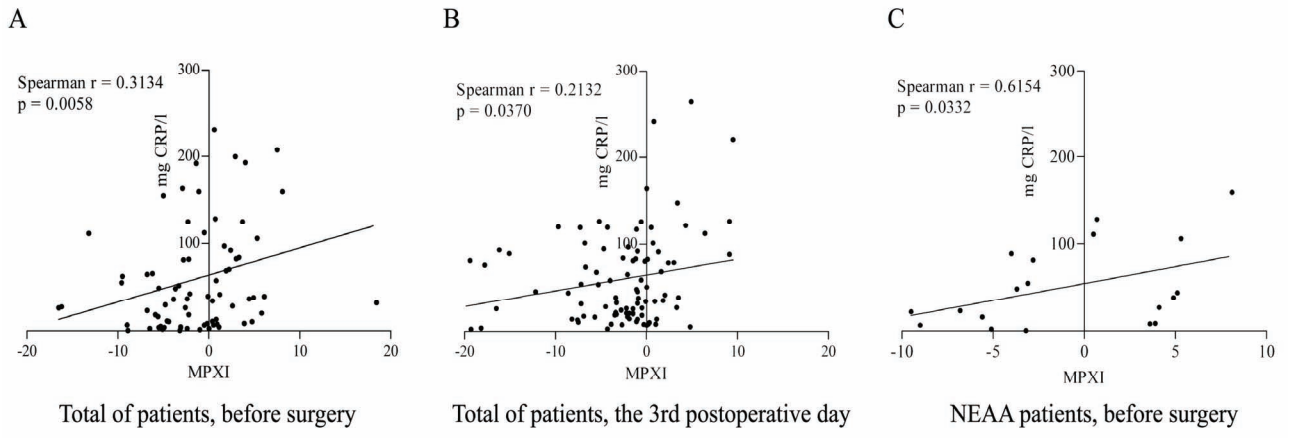


**Fig. 3 – A) Correlation between MPXI and CRP before surgery in the total AA patients regardless of intraoperative finding, showing a highly significant positive relationship; B) On the 3rd day after surgery, a statistically positive significance is present again in the total AA patients regardless of intraoperative finding; C) A statistically significant correlation between the MPXI and CRP before surgery in the NEAA group.**
**For abbreviations see under Table 1.**

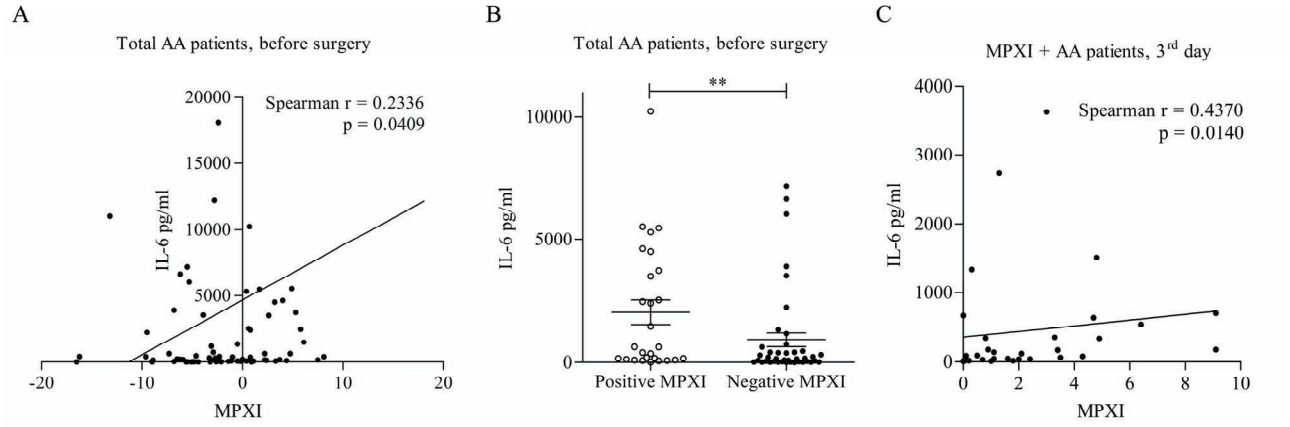


**Fig. 4 – A) Correlation between the MPXI and IL-6 in the total acute appendicitis (AA) patients before surgery, showing a statistically significant positive relationship; B) Comparison between the patients with the positive and the patients with the negative MPXI in the total AA patients, showing a significantly higher IL-6 values in the patients with the positive MPXI, before surgery; C) Correlation between the MPXI and IL-6 in the AA patients with the positive MPXI values, showing a statistically significant positive relationship in the samples taken on 3rd postoperative day. For abbreviations see under Table 1.**

*The neutrophil myeloperoxidase index correlate positively with serum interleukin-6 values*

The MPXI showed a significant positive correlation with the serum IL-6 values in the total of AA patients in similar manner as with the CRP values. Namely, the MPXI showed a significant positive relationship with the serum IL-6 values in the total of AA patients in the samples taken before surgery [Spearman's $r = 0.2336$; $p = 0.0409$ (Figure 4A)]. Next, regarding the MPXI, we stratified the patients into two groups: the group of patients with the positive MPXI and the group of patients with the negative MPXI values. When compared, the group of patients with the positive MPXI showed the significantly higher IL-6 values than the group of patients with the negative MPXI values [2041 ± 2563 vs. 917.5±1842; $p = 0.0088$ (Figure 4B)]. When we tested the samples obtained on the 1st as well as on the 3rd postoperative day, we did not find neither a significant correlation between the MPXI and serum IL-6 nor the statistical differences of IL-6 mean values between the MPXI positive and MPXI negative groups. However, when we analyzed the patients with the positive and negative MPXI separately, we found a significant positive correlation between the MPXI and IL-6 values in the samples taken on the 3rd postoperative day [Spearman's $r = 0.4370$; $p = 0.0140$ (Figure 4C)].

## Discussion

In this prospective study, which included a total of 117 of AA patients, the MPXI values were analyzed in regard to both, the intraoperative finding and that from the time elapsed since the onset of symptoms to surgical intervention. The correlations of MPXI values with the PAS, CRP and IL-6 were evaluated as well. Consecutive measurements were performed at three time points: the baseline measurement (before surgery), at the 1st and at the 3rd day after surgery. In regard to the intraoperative finding and subsequent stratification of patients into the three mentioned groups, the lowest MPXI values were recorded in the UAA group at all three time points of measurement. Further stratification of patients in regard to the duration of symptoms before surgery, revealed a very interesting observation. Namely, in the group of patients with the symptoms duration less than 24h, in the samples taken before surgery the MPXI values were lowest in the UAA group again, and that trend was maintained in the subsequent measurements. On the contrary, when the symptoms lasted longer than 24 hours, the differences between the groups were lost in samples taken before surgery as well as in the samples on the 3rd postoperative day. The study also showed the significant positive correlation of MPXI with both, CRP and IL-6, but not with the PAS.

In the diagnosis of AA, different biomarkers are used together with clinical examination and clinical history data, especially in case of diffucult diagnosis, as in children [8]. No sufficiently specific and sensitive biomarker for the AA has been found so far [9, 10]. The present study aimed to investigate a possible relationship of MPXI – a parameter that can be quickly and inexpensively assesed – with the diagnosis and clinical course of AA, as well as with some of accepted AA biomarkers and scoring systems, such as the CRP and PAS respectively.

MPO is synthesized during the myeloblast and promyelocyte stage of neutrophils maturation and its production ceases at the promyelocyte to myelocyte stage.

Degranulation decreases intracellular MPO content and activated neutrophils have lower level of MPO in comparison with non-activated or immature ones [11–13]. Some specific patterns of MPXI change were reported, such as MPXI increase in myeloid leukemia [14, 15], megaloblastic anemia [16, 17] and some bacterial infections, the low MPXI in bacterial sepsis and unchanged in viral infection and tuberculosis [18]. In addition, the MPXI is useful as independent biomarker for diagnosis and follow-up of ischemic heart diseases [19]. However, there are limited data in the literature regarding the MPXI changes in the AA patients. In the study which in-

cluded 105 patients with the AA, Kim et al. [20] did not find a significant difference in the MPXI values between the complicated and uncomplicated AA . Our data showed the significantly lower MPXI values in the patients with the uncomplicated AA when compared with the patients with complicated and the patients without and/or the early AA. In addition, the significant differences in the MPXI values recorded between the groups in the first 24 hours of onset of symptoms, may suggest a greater usefulness of MPXI as a biomarker in the early established suspicion of AA. In our patients, the lower MPXI values in the uncomplicated appendicitis were interpreted as a greater rate of activation and degranulation of mature neutrophils (less MPO content). Absence of gangrene and perforation in these cases could be explained by and effective inflammatory process still kept under control by the immune system.

The PAS is sensitive and specific in the clinical assessment of AA [21]. However, in our study, there was no significant correlation between the MPXI and PAS. We did not find a significant correlation of MPXI neither with the overall PAS nor with the single parameters of this score, including the absolute number of leukocytes and the percentage of neutrophils. Additionaly, in our previous article we showed the lack of significant correlation between the MPXI and neutrophil-to-lymphocyte ratio (NLR) [22].

CRP is very reliable biomarker for cytokine-mediated response in AA [23]. A significantly positive correlation between the MPXI and CRP, which was demonstrated in this study, implied an observation which was contradictory to a certain extent – meaning that the patients with the lowest MPXI (presumably, the patients with the highest degree of neutrophils degranulation), at the same time had the lowest CRP values (considered as a reliable marker of inflamation). The synthesis of CRP was under control of IL-6, secreted mainly by mononuclear phagocytes, vascular endothelium or T-lymphocytes [24]. When we tested the relationship between the MPXI and IL-6, we found a significant, positive correlation between them as well as the significantly higher values of IL-6 in the patients with the positive MPXI. Taken together, we speculate that the appropriate neutrophil's degranulation had efficiently controlled and limited local inflammation, and prevented early systemic response in the AA patients (i.e., the secretion of IL-6 and subsequent synthesis of CRP). In addition, the lowest MPXI values in the UAA group with a statistical significance in comparison with the NEAA and CAA groups, could suggest, on the one hand, that the unnecesary neutrophils degranulation did not happend without need (the NEAA group), and, on the other hand, that the appropriate neutrophil's reaction gave rise to the favorable clinical course (in comparison with the CAA group). There are the data which suggest that the gangrenous and phlegmonous appendicitis are different entities with divergent immune regulation and that skewing of the immune response toward Th-17 type could result in the development of gangrenous – complicated AA [25]. Possible influence of MPO on the immune response skewing should be tested in the AA patients, since the immunomodulatory properties of this enzyme was described [26].

## Conclusion

In this study, the statistically significant differences in the MPXI values between uncomplicated and complicated AA before and after surgery were found, suggesting that the MPXI may be used as an informative biomarker in the follow-up of AA in children, especially in the early phase of AA. However, a wide reference range for the MPXI and individual differences in the values of MPXI in the healthy children, generate difficulties for its use for the initial diagnosis. The described relationship between the MPXI, CRP and IL-6 speaks in favor of tight and balanced connection between the local inflamation and systemic acute phase response in AA. The evaluation of MPXI in a combination with other parameters for assessing the development and immune response during AA, should be tested by further investigations.

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

ORIGINAL ARTICLE

UDC: 616.61-006
https://doi.org/10.2298/VSP170629003T

# Ultrastructural characteristics of primary renal epithelial tumours with granular oncocytic cytoplasm

## Ultrastrukturne karakteristike primarnih epitelnih tumora bubrega sa granuliranom-onkocitnom citoplazmom

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**Abstract**

**Background/Aim.** Ultrastructural analysis of tumours has shown many common characteristics of certain neoplasms, as well as their specificities. Primary renal epithelial tumours with granular oncocytic cytoplasm is a very heterogeneous group in their histological origin and biological behaviour, which results in a difference in treatment and prognosis of the disease, making accurate morphological diagnosis necessary. The aim of this study was to determine ultrastructural similarities and differences among primary renal epithelial tumours with granular oncocytic cytoplasm. **Methods.** The analysis of archival and routine material in the archives of the Department of Pathology, University Hospital in Plzen, Czech Republic, as well as archival and routine material in the Centre for Pathology and Histology, Clinical Centre of Vojvodina in Novi Sad, discovered 346 primary renal epithelial tumours with granular oncocytic cytoplasm and divided them into 5 groups: 1) renal oncocytoma (RO) (234 tumours), 2) oncocytic papillary renal cell carcinoma (O-PRCC) (12 tumours), 3. sporadic renal hybrid oncocytic/chromophobe tumour (HOCT) without evidence of Birt Hogg Dubé syndrome (BHD) (14 tumours), 4) chromophobe renal cell carcinoma (ChRCC) (21 tumours) and 5) granular renal cell carcinoma (RCC) [64 tumours + 1 clear cell RCC (CRCC) with hyaline globules]. Ultrastructural analysis of tumour cells at the subcellular level was done using electron microscope (Philips electron microscope TEM 208) at the Department of Pathology, University Hospital in Plzen, Czech Republic. Cellular organelles and pigments were evaluated in 5 tumours from each group according to the simple random sample principle with a total of 30 analysed tumours. **Results.** In all analysed primary renal epithelial tumours with granular oncocytic, cytoplasm dominant organelles were mitochondria. Specific ultrastructural characteristics of RO were round mitochondria with lamellar cristae, whereas ChRCC had numerous typical cytoplasmic microvesicles 100–700 nm large and mitochondria with tubulovesicular, lamellar and circular cristae. Ultrastructural specificity of hybrid tumours were rare microvesicles and numerous mitochondria of O-PRCC mitochondria with lamellar cristae and small intracytoplasmic vesicles, 100–200 nm large, and of granular RCC, in addition to mitochondria, also glassy hyaline globules (GHG). **Conclusion.** Ultrastructural analysis indicates mitochondria as the dominant organelle in the analysed tumours. Electron microscopy showed specificities, i.e., differences in appearance of cristae, presence and size of vesicles as well as deposition of pigment in and out of cytoplasm and glassy hyaline globules.

**Key words:**
**kidney neoplasms; microscopy, electron; diagnosis, differential; mitochondria; cytoplasmic granules.**

**Apstrakt**

**Uvod/Cilj.** Ultrastrukturnom analizom tumora uočene su mnoge zajedničke osobine nekih neoplazmi, ali i specifičnosti. Primarni tumori bubrega sa granuliranom–onkocitnom citoplazmom su veoma heterogena grupa po svom histološkom poreklu i biološkom ponašanju, što rezultuje različitim u terapiji i prognozi bolesti, zbog čega je neohodna precizna morfološka dijagnostika. Cilj rada bio je određivanje ultrastrukturnih sličnosti i razlika između primarnih epitelnih tumora bubrega sa granuliranom-onkocitnom citoplazmom. **Metode.** Analizom arhivskog i rutinskog materijala arhive Departmana za patologiju, Fakultetske bolnice u Plzenu, Republika Češka i Centra za patologiju i histolo-

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giju, Kliničkog centra Vojvodine u Novom Sadu, pronađeno je 346 primarnih renalnih epitelnih tumora sa granuliranom-onkocitnom citoplazmom koji su razvrstani u 5 grupa: 1. renalni onkocitomi (RO) (234 tumora); 2. onkocitni papilarni karcinomi bubrega (O-PRCC) (12 tumora); 3. sporadični hibridni onkocitno/hromofobni tumori bubrega (HOCT) van Birt Hogg Dubé sindroma (BHD) (14 tumora); 4. hromofobni karcinomi bubrega (ChRCC) (21 tumor) i 5. granulirani karcinomi bubrega (RCC) [64 tumora + 1 *clear cell* RCC (CRCC) sa hijalinim globulama]. Vršena je ultrastrukturna analiza tumorskih ćelija, na subcelularnom nivou, elektronskim mikroskopom (Philips elektronski mikroskop TEM 208), Departmana za patologiju, Fakultetske bolnice u Plzenu, Republika Češka. **Rezultati.** U svim analiziranim primarnim epitelnim tumorima bubrega sa granuliranom-onkocitnom citoplazmom dominatne organele bile su mitohondrije. Specifične ultrastrukturne osobine za RO bile su okrugle mitohondrije sa lamelarnim kristama, za ChRCC brojne tipične, citoplazmatske mikrovezikule, veličine 100–700 nm i mitohondrije sa tubulovezikularnim, lamelarnim i cirkularnim kristama. Ultrastrukturna specifičnost za hibridne tumore bile su retke mikrovezikule i brojne mitohindrije, za O-PRCC mitohondrije sa lamelarnim kristama i male intracitoplazmatske vezikule veličine 100–200 nm, a za granulirane RCC pored mitohondrija i „glassy" hijaline globule (GHG). **Zaključak.** Ultrastrukturna analiza ukazuje na mitohondrije, kao dominatnu organelu u analiziranim tumorima. Elektronskom mikroskopijom uočene su i specifičnosti, odnosno razlike u izgledu krista, prisustvu i veličini vezikula, kao i deponovanje pigmenta u i van citoplazme, te "glassy" hijalinih globula.

**Ključne reči:**
**bubreg, neoplazme; mikroskopija, elektronska; dijagnoza, diferencijalna; mitohondrije; citoplazmatske granule.**

## Introduction

In 1931 Ruske and Knoll constructed the first electron microscope which was used for ultrastructural analysis of different tissues [1]. The aim of ultrastructural tissue analysis is to detect the smallest cytological characteristics of tissue and characteristics at the subcellular level (presence and appearance of organelles). Normal cells of certain tissues as well as tumour cells of different neoplasm have their specificities, but also common characteristics [1, 2].

As a differential diagnostic problem in daily histopathological diagnostics of a uropathologist, a group of primary renal epithelial tumours with granular oncocytic cytoplasm stands out. This group is very heterogeneous in its histological origin and biological behaviour, which results in a difference in treatment and the prognosis of the disease. An accurate histopathological diagnosis of this group of tumours contributes significantly to the diagnostics and treatment of patients with the aforementioned types of tumours. In some cases, after standard pathohistological analysis, immunohistochemical staining, and molecular-genetic analyses, a definitive diagnosis of the type of renal epithelial tumours with granular oncocytic cytoplasm cannot be made. In that case, ultrastructural analysis of the tumour, by means of electron microscope, can provide substantial assistance, point out some ultrastructural tumour specifics and direct us towards the correct diagnosis.

Renal oncocytoma (RO) is a benign renal epithelial tumour, representing approximately 4%–9% of all renal tumours [3, 4]. Electron microscopy shows round nuclei and cytoplasm filled with mitochondria, generally exhibiting lamellar cristae [3, 4].

Renal hybrid oncocytic/chromophobe tumour (HOCT) appears in patients with the Birt Hogg Dubé (BHD) syndrome, or without evidence of it (sporadic). This tumour can be associated with renal oncocytosis, also within the aforementioned syndrome. HOCT without evidence of BHD syndrome, or renal oncocytosis is very rare. Ultrastructurally, as well as histologically, it can have characteristics of two components: chromophobe carcinoma and oncocytoma [3–10].

Papillary/chromophilic renal cell carcinoma (PRCC) is the second most frequent RCC with incidence of 11%–15%. Its five-year and ten-year survival rates are higher than in clear-cell RCC (CRCC), although some studies suggest that there is no difference between CRCC and PRCC. PRCC with oncocytic cytoplasm and low-grade nuclei are referred to as oncocytic, PRCC having a biological behaviour of type 1 PRCC. Ultrastructurally, beside mitochondria with glycogen granules, they have basal infoldings [5–7, 11–17].

Chromophobe renal cell carcinoma (ChRCC) is the third most common when talking about the subtypes of RCC. It was first published in 1985 by Thoenes et al. [16] and other authors [5–7, 17–19]. Its prognosis is much better than the one for CRCC, with indolent course of disease, and some studies give it even better prognosis than for PRCC [3, 4]. Electron microscopy shows cytoplasmic vesicles and abundance of mitochondria, often with tubulocystic cristae.

CRCC is characterized by the von Hippel-Lindau gene mutation (3p25-26) or chromosome 3p loss. CRCC is the most aggressive and most common histologic type of carcinoma, with five and the ten-year survival rates of 75%, and 62% respectively. Eosinophilic variant of clear cell renal cell carcinoma/granular cell RCC occurs as part of conventional CRCC, as larger and smaller areas of cells with eosinophilic cytoplasm, or in a pure form. Differentiating granular RCC from ChRCC and RO is one of the most difficult differential diagnostic problems in renal pathology. Unlike chromophobe cell carcinoma, which is an indolent, and RO, which is a benign tumour, granular RCC is a very aggressive neoplasm. Electron microscopy shows mitochondria as the dominant organelle and a fewer number of microvesicles [16, 17, 20–23].

## Methods

The study was retrospective and prospective. It included the patients with primary renal epithelial tumours with granular oncocytic cytoplasm, after partial, or total nephrectomy. Routine and archival materials were used, lo-

cated in the computerised archives of the Department of Pathology of the University Hospital in Plzen, Czech Republic, as well as archival and routine materials of the Centre for Pathology and Histology, Clinical Centre of Vojvodina in Novi Sad, Republic of Serbia.

In the period from January 2010 to December 2015, after examination of both archives and daily routine diagnostics, the study included 346 primary renal epithelial tumours with granular oncocytic cytoplasm that were classified into 5 groups: 1) RO (234 tumours), 2) oncocytic PRCC (O-PRCC) (12 tumours), 3) sporodic HOCT (14 tumours), 4) ChRCC (21 tumours), and 5) granular RCC (64 tumours + 1 CRCC with glassyhyaline globules).

The ultrastructural analysis of tumour cells at the subcellular level was done using electron microscope (Philips electron microscope TEM 208), at the Department of Pathology, University Hospital in Plzen, Czech Republic, with cellular organelles and pigments evaluated in 5 tumours from each group according to the simple random sample principle, with the total of 30 tumours.

The small pieces of wet and formalin-fixed tissue of about 1 mm² were cut into drops of fixative into small pieces and then transferred to the fixative (4% solution of glutaraldehyde in 0.1M Na-cacodylate buffer pH 7.3) where they were held for two hours at a temperature of 4°C. After two to three rapid changes in Na-cacodylate buffer, the material was postfixed in 1% solution of OsO4 in 0.1M Na-cacodylate buffer at 4°C, for 2 hours. This was followed by two to three rapid changes in Na-cacodylate buffer and the samples were stored overnight in a 4% uranyl acetate solution, in order to increase the contrast of the material. The segments were then put through a series of alcohols (25%, 50%, 70%, 80%, 90% and 100%), followed by dehydration and "illumination" by keeping them in propylene oxide two times for 10 minutes. The sampling in resin was done at room temperature, through three different mixes of propylene oxide and Epon resin (3:1, 1:1, 1:3). This way, samples were put into pure Epon resin and held in it overnight at room temperature. After embedding in the plastic pellets, the resin was polymerized at 60°C for three days. After completed polymerization samples were cut on LKB ultramicrotome III, with the glass and diamond knives. Sections up to 1 micron thick- semi-fine sections were cut first. The sections were transferred to glass slides and stained with aqueous toluidine blue and borax solution, over a flame, at a temperature of 80°C. The molds were cut with a diamond knife on a LKB ultramicrotome III, to the thickness of the section of 30–50 nm, placed on the copper-meshes coated with paraffin. Subsequently, they were positively stained for 20 minutes with 5% uranyl acetate solution, and then washed with redistilled water, and air-dried. Dry sections were stained with Reynolds lead citrate solution for 10 minutes, then washed and dried again.

The analysis of stained sections was performed using the Philips electron microscope TEM 208, at the Department of Pathology, University Hospital in Plzen, Czech Republic.

**Results**

After the histopathological evaluation using the standard hematoxylin and eosin (HE) staining, immunohistochemical staining and molecular genetic analyses, tumours were classified into 5 groups and ultrastructurally analysed.

Using electron microscope, the ultrastructure of RO was analysed, with some having small cell components and/or pseudorosettes. Figure 1A shows the ultrastructure of small cell variant of RO. Classical oncocyte had typical cytoplasm packed with numerous round mitochondria, with lamellar cristae. Mitochondria were also the most common organelles in small cells, but in considerably smaller number compared to classical tumour component. Microvilli and plasmalemma elements could not be observed in either cell types. The ultrastructural analysis of the two RO with vascular invasion showed oval nuclei with small nucleoli. Cytoplasm of tumour cells was packed with predominantly lamellar mitochondria. Cytoplasmic lumens, covered with short microvilli could be observed in some places. Luminal surface of oncocytic cells was also coated with microvilli.

The findings were consistent in all cases of renal hybrid oncocytic-chromophobe tumour analysed using the electron microscope. Neoplastic cells had numerous mitochondria, of different sizes. Rare microvesicles with amorphous lamellar content were also detected. Tumour cells with abundant microvesicles in their cytoplasm were not noticeable. Nuclei were mostly round and with hardly noticeable nucleoli. Small intracytoplasmic tubules covered in microvilli were observed in one case.

The ultrastructural analysis of O-PRCC showed cytoplasm filled with numerous mitochondria with lamellar cristae (Figure 1B). Other organelles were present separately. Intracytoplasmic vesicles were 100 to 200 nm in diameter and granular endoplasmic reticulum was rarely noticeable.

Tumour cells of renal chromophobe carcinoma showed weaker bond-forming in outer lamina. Two main intracellular components were detected: typical cytoplasmic vesicles, commonly observed in ChRCC, 100–700 nm in size (Figure 1C), and mitochondria with tubulovesicular, lamellar and circular cristae.

In addition to preserved mitochondria, there were also the degenerated ones with alternately oriented internal cristae. Some tumour cells contained dark, electronically dense pigment pellets, that corresponded to the brown pigment observed under the light microscope. The aforementioned pigment pellets were round to polygonal (Figure 1D).

In addition, some tumours had vesicles containing tiny beads of the same dark, electronically dense material as the granules. This material melted and built larger pigment pellets. Melanosomes and neurosecretory pellets in neoplastic cells could not be observed in any of the analyzed cases.

Mitochondria were also dominant organelles in granular CRCC. Ultrastructurally smallest GHG was like an amorphous secretion within a cisterna of granular endoplasmic reticulum (Figure 1E). Ultrastructurally larger GHG were in the form of globules, filled with amorphous material, inside cytoplasm or extracellularly.

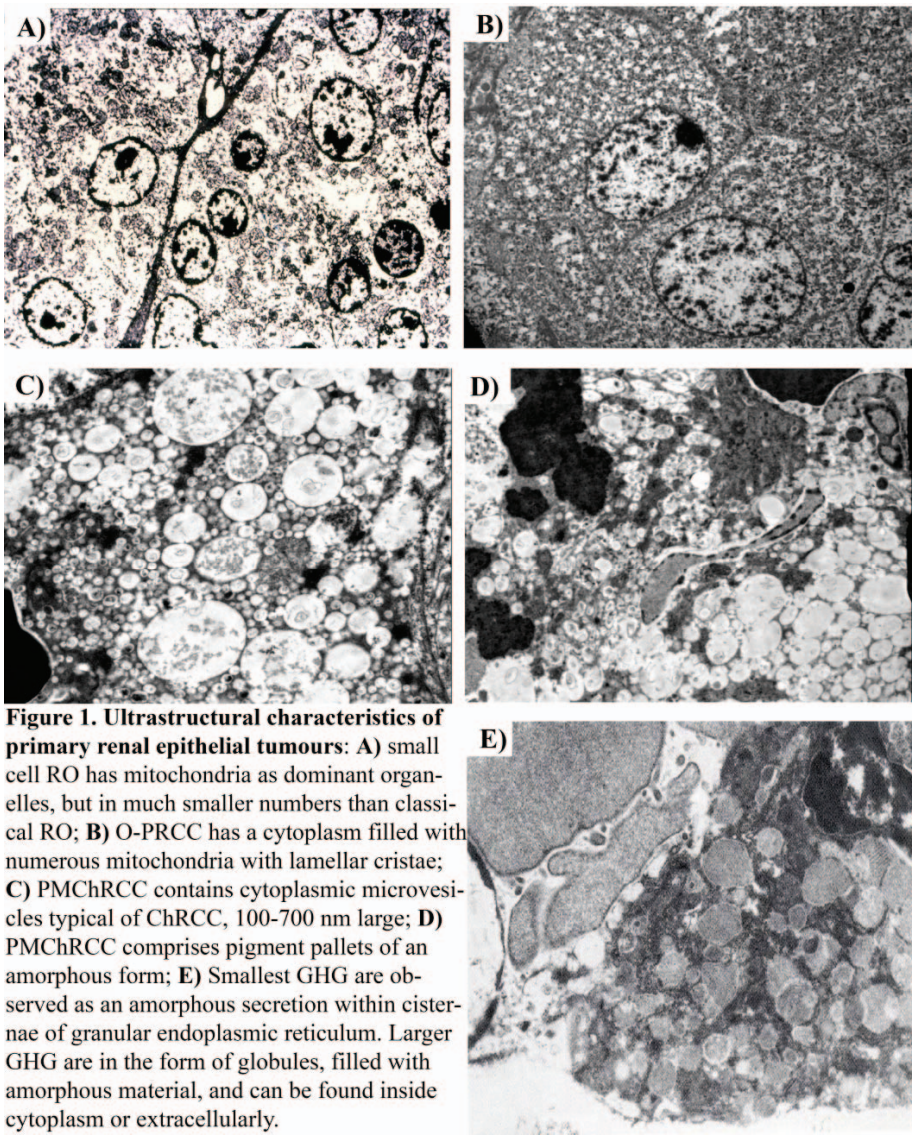


**Figure 1. Ultrastructural characteristics of primary renal epithelial tumours**: **A)** small cell RO has mitochondria as dominant organelles, but in much smaller numbers than classical RO; **B)** O-PRCC has a cytoplasm filled with numerous mitochondria with lamellar cristae; **C)** PMChRCC contains cytoplasmic microvesicles typical of ChRCC, 100-700 nm large; **D)** PMChRCC comprises pigment pallets of an amorphous form; **E)** Smallest GHG are observed as an amorphous secretion within cisternae of granular endoplasmic reticulum. Larger GHG are in the form of globules, filled with amorphous material, and can be found inside cytoplasm or extracellularly.

**RO – renal oncocytoma; O-PRCC – oncocytic papillary renal cell carcinoma; PMChRCC – psammoma chromophobe renal cell carcinoma; GHG – glassy hyaline globules.**

## Discussion

Under the electron microscope, classical oncocytoma showed typical picture of small nuclei with nucleoli and cytoplasm, with densely packed round mitochondria with lamellar cristae, which is in accordance with other literature references [24–27]. In small cells, mitochondria were also the most prevalent organelles, although the number of mitochondria was significantly lower than in the traditional components. Ultrastructurally, microvesicles with amorphous lamellar content were barely present. These structures were typically abundantly present in neoplastic cells of the conventional ChRCC, however, they were often damaged due to inadequate fixation. Microvesicles that can be seen in almost all chromophobe RCC and are rarely described in RO, were not detected in our cases [28].

Ultrastructurally, cytoplasm of renal hybrid oncocytic-chromophobe tumour also had numerous mitochondria of different sizes, with rare microvesicles with amorphous lamellar content, and it could differentiate it from ChRCC, with which it had the greatest morphological similarity [29]. Nuclei were mostly round, with very rarely observed nucleoli, and the presence of small intracytoplasmic tubules covered with microvilli, in one case, could also facilitate the diagnosis and indicated this rare type of tumour with granular oncocytic cytoplasm.

The cytoplasm of O-PRCC tumour cells was packed with large mitochondria that had lamellar cristae, such as those seen in RO. This finding has already been described by Erlandson et al. [27], in the example of two cases of papillary RCC.

In terms of differential diagnosis, psammoma ChRCC (PMRCC) is a challenging tumour. Considering its more indolent behavior compared to other renal cell carcinomas, it is important to distinguish ChRCC from RO, which is sometimes very difficult. In general RO shows a wide spectrum of

morphological images. RO polygonal tumour cells are typically uniform, usually with abundant eosinophilic cytoplasm filled with mitochondria, but without vesicles whose diameter is, in case of ChRCC, 100–700 nm [20, 27, 30]. The psammoma bodies and calcifications are often present in both RO and PMChRCC. Dark brown pigment and architectural features we listed as well as its morphological diagnostic characteristics should be helpful in diagnosing PMChRCC.

Glassy hyaline globules can occasionally be seen in tumours of several organs, brain, liver, breast, lung, adrenal gland [31–33] and gonads (tumours of germ cell and non-germ cell origin). Even if hyaline globules in these organs have similar appearance, they probably represent heterogeneous structures with different histogenesis. Our experience is that GHG is rarely seen in low-grade papillary RCC. These tumours are practically easily confused with granular RCC. GHG of similar appearance to those that we described in our study have previously been described in RCC [34, 35]. Datta [36] published a case of RCC with globules of ultrastructural appearance similar to the appearance of structures with osmiophilic, dense aggregates, i.e., fine granules without the membrane, which were closely arranged in relation to the strips of granular endoplasmic reticulum. In other paper, describing hyaline globules in RCC, Jagirdar et al. [35] made a parallel between them and Mallory bodies in the liver. We do not know of any systemic study that described the extent of GHG in different types of renal carcinoma. In our work, it was observed that GHG were specifically present in granular and mixed type of clear/granural cells of RCC. Globules were not detected in cases of ChRCC and RO. This finding may potentially be significant, for it is sometimes very difficult to distinguish between the two types of tumour, ChRCC and RO. It is believed that in general, the so-called metastasising RO represents probably misdiagnosed granular RCC, or chromophobe RCC [36, 37].

The most commonly diagnostic methods employed to find differences between renal tumours with granular-oncocytic cytoplasm, the standard HE staining, histochemical, immunohistochemical and sometimes molecular genetics, often do not provide enough criteria to give additional information in establishing the diagnosis. It seems that the most effective one is electron microscopy, but it is still a quite expensive and time-consuming method. Correct diagnosis is very important, as the prognoses for these three types of tumours are significantly, dramatically different. Chromophobe RCC is a relatively indolent type of carcinoma, RO is a benign neoplasm [21, 23], and granular RCC is an aggressive type of tumour [16, 21–23]. Identification of GHG in granular RCC can serve as a potentially valuable and reliable morphological finding in distinguishing these tumours. Identification of GHG is easy and reproducible. It can be used to diagnostically separate granular and mixed type of clear/granular cell RCC from chromophobe renal cell carcinoma and renal oncocytomas.

Bonsib et al. [38] described globular filamentous bodies in RO that should not be identified as, or mistaken for GHGs. He detected these globules in 16 out of 20 cases of RO, but they were not observed in 35 renal cell carcinomas (clear cell, granular cell, papillary and chromophobe RCC) [39, 40]. The aforementioned globular filamentous bodies differ significantly from GHGs that we described. Ultrastructurally, they look like discreet round to oval cytoplasmic foci, poor of mitochondria, containing collections of intermediate filaments, occasionally mixed with lipid drops, lysosomes, mitochondria, smooth endoplasmic reticulum or lamellae of Golgi apparatus. The described structures probably belong to the family of similar bodies seen in a variety of tumours, named after globular filamentous bodies, by Ghadially [39]. Under the light microscope, unlike GHG in granulated RCC, they are easily detectable and in our opinion should not be morphologically mistaken for GHG.

Another type of hyaline globules in RCC and oncocytoma was described by Gatalica et al. [40]. They cite the PAS-positive spherical accumulations of amorphous materials with extracellular localisation. Ultrastructurally, hyaline globules in their case were constructed of materials of the basal membrane. GHG differ from the aforementioned hyaline globules (HG), in that they are frequently intracellularly localised, and ultrastructurally, they originate from cisternae of granular endoplasmic reticulum, such as the amorphous secretion [39–41].

Pathohistological classification of renal tumours with granular-oncocytic cytoplasm is not always simple and routine, because in such cases the diagnosis should include standard pathohistological analysis, immunohistochemical stainings, molecular genetics i.e., cytogenetics and ultrastructural analysis, as was cited and done by other authors in their studies [26, 42–47].

## Conclusion

In RO mitochondria represent the most prevalent organelles, although the number of mitochondria is significantly lower in oncoblasts, the small cell components, than in a traditional, i.e., oncocytes. Ultrastructurally, small cell RO with pseudorosettes also have numerous mitochondria of different sizes. O-PRCC contains numerous large mitochondria with lamellar cristae, just like renal oncocytomas. PMChRCC contains cytoplasmic vesicles typical of ChRCC, and mitochondria with tubulovesicular, lamellar and circular cristae. The presence of GHG in granular RCC can serve as a potentially valuable and reliable morphological finding in distinguishing this tumour from chromophobe RCC and RO. GHG are present in "pure"granular RCC and the mixed type of clear/granular cell RCC and are related to the poor differentiation, necrosis and bleeding. GHG are not specific for RCC, but can be useful when we encounter metastatic carcinoma of unknown origin, since the presence of GHG in the background of eosinophil cells, granular cytoplasm, or metastatic carcinoma always cause suspicion of renal carcinoma. Electron microscopy, as an expensive and time-consuming method, is the last resort in diagnosis of renal epithelial tumours with granular oncocytic cytoplasm. It provides considerable assistance when a definitive diagnosis cannot be made after the standard histopathological diagnosis, immunohistochemistry and molecular genetic analyses, hence it should not be *a priori* dismissed as part of diagnostic procedure.

**Acknowledgement**

The authors are very grateful to professor Michal Michal, Department of Pathology, University Hospital in Plzen, Czech Republic, who unselfishly allowed us to use the material from their archives and provided a big professional, technical and material support in writing this and other papers.

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Received on June 29, 2017.
Revised on September 27, 2017.
Accepted on January 11, 2018.
Online First January, 2018.

ORIGINAL ARTICLE


UDC: 364.642:364.63]:159.922.8
https://doi.org/10.2298/VSP170514009S

# The association betweeen conative functioning of adolescents exposed to intimate partner violence and family dimensions of cohesion and adaptibility

## Povezanost između konativnog funkcionisanja adolescenata izloženih interpartnerskom nasilju i porodičnih dimenzija kohezivnosti i adaptabilnosti

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**Abstract**

**Background/Aim.** Childhood exposure to various types of emotional, physical and sexual abuse in intimate partner violence (IPV) families is associated with difficulties in emotional and social adjustment, including conduct problems, internalized and externalized symptoms. The aim of this study was to determine the relationship between intimate partner violence and: family system functioning (cohesion and adaptability), conative functioning of adolescents and risk of psychopathological symptomatology development. The specific aim of this study was to establish the mental hygiene and preventive measures in order to reduce the negative consequences of growing up in the IPV families. **Methods.** The study was done on a sample of 308 adolescents, aged 15–18 years, divided into the IPV group (n = 68 adolescents growing up in families with the IPV and exposed to IPV in which the violence was reported and processed), and the control group (n = 240 adolescents coming from families in which there was not found any type of violence or psychosocial pathology on the basis of the results obtained on the Conners' Parent Rating Scale–Revised (CPRS-R) Questionnaire. The Faces III scale of measurement was used for measuring the dimensions of family functioning, and Cybernetic model of conative dimensions of personality (CON-6) for conative functioning of adolescents. The data was processed by using the discriminate and linear regressive analysis. **Results**. The adolescents growing up in the families with the IPV and exposed to IPV showed the statistically significant differences ($p < 0.01$) in conative functioning: psychosomatic β = -.509, anxiety β = -.393, aggressive β = -.398, dissociative β = -.509 and adaptive personality reactions β = -.455, as compared to the control group. There was 32.35% of adolescents exposed to IPV who showed the pronounced pathological values regarding social-adaption reaction, 23.53% regarding pathological anxiety and 23.53% dissociative reactions. The greatest negative relationship was found between intimate partner violence and family dimension of cohesion (β = -0.605, $p < 0.01$). **Conclusion**. Adolescents growing up and being exposed to the intimate partner violence were significantly associated with changes in the conative functioning and risk of externalized and internalized symptoms development in socially-adaptive, anxiety and dissociative reactions and the need to introduce the preventive mental-hygienic measures. The mediator between IPV and conative functioning of adolescents was family cohesion.

**Key words:**
**behavior; mental disorders; adolescent; family; violence; adaptation, psychological; impulsive behavior.**

**Apstrakt**

**Uvod/Cilj.** Izloženost dece različitim oblicima emocionalnog, fizičkog i seksualnog zlostavljanja u porodicama sa partnersko porodičnim nasiljem (PPN) povezana je sa teškoćama u emocionalnom i socijalnom prilagođavanju, uključujući i probleme ponašanja, internalizovane i eksternalizovane simptome. Cilj istraživanja bio je da utvrdi povezanost PPN sa funkcionisanjem porodičnog sistema (kohezivnost i adaptabilnost), konativnim funkcionisanjem adolescenata i rizikom za razvoj psihopatološke simptomatologije. Specifični cilj studije bio je utvrđivanje mentalno-higijenskih i preventivnih mera radi smanjenja negativnih posledica odrastanja u porodicama sa nasiljem. **Metode.** Istraživanje je sprovedeno na uzorku od 308 adolescenata, uzrasta od 15–18 godina, podeljenih u IPV grupu (n = 68 adolescenata koji su odrastali u porodicama sa partnerskim nasiljem i izloženi partnerskom nasilju, koje je prijavljeno i procesuirano i kontrolnu grupu [n = 240 adolescenata iz porodica u kojima upitnikom *Conners' Parent Rating Scale–Revised* (CPRS-R) nije utvrđen bilo koji oblik nasilja ili psihosocijalne patologije]. Skala Faces III korišćena je za merenje dimenzija porodičnog funkcionisanja, a Kibernetički model konativnih dimenzija ličnosti (KON-6) za konativno funkcionisanje ado-

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lescenata. Podaci su obrađeni diskriminativnom i linearnom regresionom analizom. **Rezultati**. Adolescenti iz porodica sa partnerskim nasiljem imali su statistički značajne razlike ($p < 0,01$) u konativnom funkcionisanju: psihosomatskim β = -.509, anksioznim β = -.393, agresivnim (β -.398, disocijativnim β -.509 i adaptivnim reakcijama ličnosti β = -.455, u odnosu na kontrolnu grupu. Ukupno, 32,35% adolescenata iz porodica sa partnerskim nasiljem imalo je izražene patološke vrednosti u socijalno-adaptivnim reakcijama, 23,53% patološku anksioznost i 23,53% disocijativne reakcije. Najveća negativna povezanost dobijena je između porodičnog nasilja i porodične dimenzije kohezivnosti (β - 0,605; $p < 0,01$). **Zaključak**. Izloženost adolescenata porodičnom nasilju značajno je povezana sa promenama konativnog funkcionisanja i rizikom od razvoja eksternalizovanih i internalizovanih simptoma u socijalno-adaptivnim, anksioznim i disocijativnim reakcijama i potrebom za uvođenje preventivnih mentalno-higijenskih mera. Medijator između porodičnog nasilja i konativnog funkcionisanja adolescenata je porodična kohezivnost.

**Ključne reči:**
**ponašanje; ponašanje, poremećaji; adolescenti; porodica; nasilje; adaptacija, psihološka; ponašanje, impulsivno.**

## Introduction

Childhood exposure to various emotional, physical and sexual abuses in intimate partner violence (IPV) families is associated with difficulties in emotional and social adjustment, including conduct problems, internalized and externalized symptoms.

This study pointed to the relationship between intimate partner violence (IPV) and family functioning problems in parenting behaviour and numerous psychosocial developmental difficulties of adolescents [1].

Family stability, homework routines, discipline and cohesiveness were associated with the internalized/externalized symptoms and they significantly predicted symptomatology. A positive relation was found between parenting behaviour and child adjustment problems [2]. Family functioning problems in parenting behaviour failed to mediate relations between maternal psychopathology and adolescent problems. Maternal psychopathology was directly associated with adolescent internalized problems. Paternal perceptions of family functioning problems mediated relations between paternal psychopathology and adolescent externalized problems [3]. The adolescents whose mothers were victims of physical IPV showed higher incidence of DSM-IV disruptive disorders and externalized behaviour problems (high risk of serious conduct problems). The adolescents who were directly exposed to physical IPV and were also victims of physical punishment by parents showed increased internalized problems. IPV affected badly children's externalized problems either directly or indirectly through physical punishment [4].

Adolescent exposure to IPV was associated with aggression [5], impulsive behaviour, attention deficit disorder (ADD), attention deficit disorder/hyperactivity disorder (ADHD), conduct disorder (CD) – authority issues, disorders of habits (thumb sucking, night terrors), hyperactivity, bulimia and delinquent behaviour [6].

Numerous studies found the association between adolescent exposure to IPV and internalized symptoms: depression, anxiety, hypersensitivity, learned helplessness and fear and acute condition [7, 8].

Childhood exposure to traumatic experience of witnessing, or being a victim of intimate partner violence showed increased incidence of somatic symptoms (SS), which might result in diminished daily functioning. Over 95% of children showed at least one SS on the child-rated measure. The children who had been victims of sexual abuse had the higher rates of SS, anxiety, depression, post-traumatic stress, dissociation and anger. Anxiety mediated the association between sexual abuse and child-rated SS [9].

Growing up in the families with various forms of violence influenced the development of expressed symptoms of post-traumatic stress disorder, due to the presence of children to murders, suicides or fights between parents. Further development of post-traumatic symptomatology in youth led to the appearance of depression, suicidality [10], anxiety [11], drug addiction (cigarettes, marijuana) and the intergenerational transmission of family violence [12].

The study examined the relationship between IPV, child abuse and neglect, other traumatic experiences and children's post-traumatic stress (PTS) symptoms, and explored the moderating role of family functioning in the aftermath of IPV with the PTS symptoms. For family functioning, the higher levels of parenting stress were associated with the higher levels of PTS symptoms [13].

An increase in the index of intimate partner violence in Serbia and its consequences on the murder rate and number of psychic difficulties of family members led to defining a "National strategy for preventing and containing violence against women in the family and intimate partner violence" [14].

## Methods

The study was conducted on a sample of 308 adolescents, aged 15–18 years [mean ± standard deviation (SD) = 16.5 ± 1.5]. There were no differences between the groups by the age ($t_{(198)} = -1.09$, non-significant). The sample consisted of two subsamples: the IPV one was made up of adolescents exposed to IPV, which was reported to and processed at the Centre for Social Work in Vranje, Serbia (n = 68/22.1%) and the control one made up of 240/77.9% adolescents coming from families in which there were not found any type of violence and social pathology using the revised CPRS-R Questionnaire. The Scale of Family Adaptability and Cohesion (FACES III) was used for measuring family functioning dimensions (cohesion, adaptability) and Cybernetic model of conative personality dimensions (CON-6) for conative functioning of adolescents.

After being acquainted with the Approval of the Ethics Committee of the Faculty of Medical Scinces, University of Kragujevac, Serbia, as regards conducting the design of study and upon getting the respective Consent by the Centre for Social Work in Vranje and Secondary School "Gimnazija Bora Stanković" in Vranje, Serbia, the respondents gave, after being acquainted with the aims of the study, written information consent for participating in the study.

The testing was conducted by psychologists and specialist teachers, who did therapeutic work with the parents and the adolescent or the adolescent only. The study was anonymous. Testing the total test material took one hour. The respondents filled out the CON-6 in 30 minutes, while answering the Questionnaire CPRS-R and FACES III lasted up to 15 min. The study was conducted during the period from 2010 to 2012.

The results for the control group of adolescents coming from the families without IPV were obtained by using the CPRS-R. It contained 20 items [15]. In this study, the reliability estimates for the scores was $\alpha = 0.94$.

Family cohesion and adaptability dimensions were measured by using the FACES III, which consisted of 20 items. The obtained results were expressed by using the raw scores and categories (cohesion: low – dissociated; mild – separated; developed – coherent, and high – intertwined; adaptability: low, moderate – flexible; developed – structured; high – rigid) [16]. In this study, the reliability estimates for the scores was $\alpha = 0.89$.

The conative dimensions, measured by the CON-6 were: reaction activity -introversion/extroversion, psychosomatic reactions (anxiety, defence reactions, aggressive reaction – response attacks, dissociative reactions, integrative reaction – social adaptation). It contained 180 items. The conative personality dimensions were expressed in the following categories: superior, above–the–average, average, below-the-average, and pathological functioning [17]. In this study, the reliability estimates for the scores were $\alpha = 0.96$.

The data analysis was performed using the software package SPSS version 11.5. Discriminative analysis was used for determining the level of pronunciation of family and conative dimensions of adolescents. The significance of differences between the samples was determined by using the $\chi^2$ test, One-Way ANOVA and *t* test. Linear regression model was used for determining the relationship between family violence, family and conative functioning of the adolescents. The level of statistical significance was less than 1% ($p < 0.01$) and 5% ($p < 0.05$).

## Results

The family dimension of cohesion was statistically larger in the functional non-violence families. The IPV families and functional non-violence families significantly ($p \leq 0.002$) differed when it comes to the level of pronunciation of adaptability dimension (Table 1).

The category of high cohesion, closeness among family members, was more pronounced with the functional non-violence families, as opposed to the families with violence ($p \leq 0.001$) (Table 2).

**Table 1**

**Family dimensions of cohesion and adaptability in the functional, non-violence families and IPV families**

| Family dimensions | Statistical parameters | Type of family | | |
|---|---|---|---|---|
| | | Functional | IPV | Total |
| Cohesion | mean ± SD | 41.050 ± 4.314 | 29.088 ± 1.616 | 35.069 ± 2.965 |
| | F* | | 157.049 | |
| | df | | 1 | |
| | *p* | | 0.000 | |
| Adaptability | mean ± SD | 29.887 ± 5.388 | 26.667 ± 6.521 | 29.943 ± 6.127 |
| | F | | 10.010 | |
| | df | | 1 | |
| | *p* | | 0.002 | |
| Total | n | 240 | 68 | 308 |

**IPV – Intimate partner violence; SD – standard deviation; *One-way ANOVA.**

**Table 2**

**Dimension categories of cohesion in the functional, non-violence and IPV families**

| Cohesion | Type of family, n (%) | | |
|---|---|---|---|
| | Functional | IPV | Total |
| Low, disunited | 0 (0.0) | 4 (5.9) | 4 (1.3) |
| Moderate, separated | 1 (0.4) | 22 (32.3) | 23 (7.5) |
| Developed, related | 62 (25.8) | 28 (41.2) | 90 (29.2) |
| High, intertwined | 177 (73.8) | 14 (20.6) | 191 (62.0) |
| Total (n) | 240 | 68 | 308 |
| $\chi^2 = 126.191$; df = 6; $p = 0.000$ | | | |

**IPV – Intimate partner violence**

The category of rigid/high adaptability was more pronounced with the IPV families than with the functional non-violence families. The functional non-violence families had the most prevalently developed adaptability, whereas 50% of IPV families had the moderate and developed adaptability dimension (Table 3).

The conative functioning of adolescents growing up in the families with IPV and were exposed to IPV statistically significantly differed from the control group ($p \leq 0.001$) in the measured dimensions: organic regulator (χ – psychosomatic reactions), defence reaction regulator [alpha–anxiety reactions, attack reaction regulator (sigma–aggressive reactions), system for coordinating regulation functions (delta–dissociative reactions), system for integrating regulation functions (etha–social im/maturity)], except for the activity regulator dimension (epsylon introversion / extroversion) (Table 4).

The study found a significant ($p \leq 0.001$) difference in the pathological functioning of conative dimensions between the adolescents exposed to family violence: in the dimension of activity-introversion/extroversion regulator; psychosomatic reactions; defence reactions/anxiety reactions; attack reactions/aggressive reactions; system for coordination of regulation functions/dissociate reactions; system for integrating regulation functions or social adaptation problems, as opposed to the adolescents from functional non-violence families (Table 5).

The regression linear analysis was used for determining the relationship of IPV with the family dimensions (cohesion and adaptability) and conative functioning of adolescents, as opposed to the adolescents from the functional non-violence families. Intimate partner family violence was significantly ($p \leq 0.001$) associated with the family dimensions and conative functioning of adolescents. The slight relationship was found between IPV and the activity dimension. The low negative relationship was found between IPV and conative dimensions: defence reactions (anxiety reactions) and defence reactions – aggressive reactions. The moderate, negative, relevant correlation was found between IPV and the system for integrating regulator functions – social immaturity, organic functioning regulator – psychosomatic reactions and system for coordinating regulator functions – dissociative reactions ($p \leq 0.001$). The mediator between IPV and the conative functioning of adolescents was family cohesion. Intimate partner family violence and adaptability were significantly, but slightly related ($p = 0.002$). The relationship between intimate partner family violence and conative functioning of adolescents was not significant in the activity dimension (epsylon–introversion/extroversion, $p = 0.260$). IPV predicted the best the development of family cohesion and conative dimension of adolescents: psychosomatic, dissociative reactions and social adaptation (Table 6).

**Table 3**

**Dimension categories of adaptability in the functional, non-violence and IPV families**

| Adaptability | Type of family, n (%) | | |
|---|---|---|---|
| | Functional | IPV | Total |
| Moderate, flexible | 44 (18.3) | 30 (44.1) | 74 (24.1) |
| Developed, structured | 178 (74.2) | 30 (44.1) | 208 (67.5%) |
| High, rigid | 18 (7.5) | 8 (11.8) | 26 (8.4%) |
| Total (n) | 240 | 68 | 308 |
| $\chi^2 = 24.319$; df = 4; $p = 0.000$ | | | |

**IPV – Intimate partner violence.**

**Table 4**

**Pronunciation of conative dimensions of adolescents from functional, non-violence and dysfunctional IPV families**

| Type of family | Statistical parametars | Epsylon | χ | Alpha | Sigma | Delta | Etha |
|---|---|---|---|---|---|---|---|
| Functional | mean | 112.88 | 47.05 | 69.91 | 82.81 | 41.92 | 54.52 |
| | SD | 14.375 | 11.348 | 18.169 | 12.214 | 9.774 | 13.257 |
| IPV | mean | 115.91 | 72.06 | 94.44 | 99.97 | 70.68 | 77.47 |
| | SD | 17.140 | 26.058 | 24.036 | 18.118 | 26.487 | 23.452 |
| ANOVA | F | 1.275 | 95.262 | 49.753 | 51.299 | 145.705 | 70.922 |
| | df | 1 | 1 | 1 | 1 | 1 | 1 |
| | *p* | 0.260 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

**IPV – Intimate partner violence; SD – standard deviation.**

**Table 5**

**Pathological functioning of conative dimensions of adolescents from functional non-violence families and IPV families**

| Pathological functioning of conative dimensions | Type of family, n (%) | | |
|---|---|---|---|
| | Functional | IPV | Total |
| Extroversion-Introversion | 4 (1.7) | 6 (8.8) | 10 (3.2) |
| Psychosomatic reactions | 2 (0.8) | 12 (17.6) | 17 (5.5) |
| Anxiety reactions | 2 (0.8) | 16 (23.5) | 18 (5.8) |
| Aggressive reactions | 4 (1.7) | 14 (20.6) | 18 (5.8) |
| Dissociative reactions | 0 (0) | 16 (23.5) | 16 (5.2) |
| Social im/maturity | 4 (1.7) | 22 (32.4) | 26 (8.4) |
| Total (n) | 240 | 68 | 308 |

**IPV –Intimate partner violence.**

**Table 6**

**Linear regression analyses of adolescent and family conative dimensions**

| Variables | R | $R^2$ | Adjustment $R^2$ | F | *p* | β | *t* test | *p* |
|---|---|---|---|---|---|---|---|---|
| Epsylon | 0.068 | 0.005 | -0.001 | 1275 | 0.266 | -0.068 | 23.127 | 0.000 |
| χ | 0.509 | 0.259 | 0.257 | 95.262 | 0.000 | -0.509 | 19.889 | 0.000 |
| Alpha | -0.393 | 0.155 | 0.152 | 49.753 | 0.000 | -0.393 | 17.962 | 0.000 |
| Sigma | 0.398 | 0.159 | 0.156 | 51.299 | 0.000 | -0.398 | 25.670 | 0.000 |
| Delta | 0.591 | 0.349 | 0.346 | 145.705 | 0.000 | -0.591 | 21.912 | 0.000 |
| Etha | 0.455 | 0.207 | 0.204 | 70.992 | 0.000 | -0.455 | 19.347 | 0.000 |
| Cohesion | 0.605 | 0.366 | 0.364 | 157.049 | 0.000 | -0.605 | 9.420 | 0.000 |
| Adaptability | 0.188 | 0.035 | -0.032 | 10.010 | 0.002 | 0.188 | 12.139 | 0.002 |

The data obtained in the study showed that IPV was significantly associated with the changes in the conative functioning and risk of development of internalized and externalized psychosomatic symptoms of adolescents, where the mediator was decreased family cohesion (Figure 1).

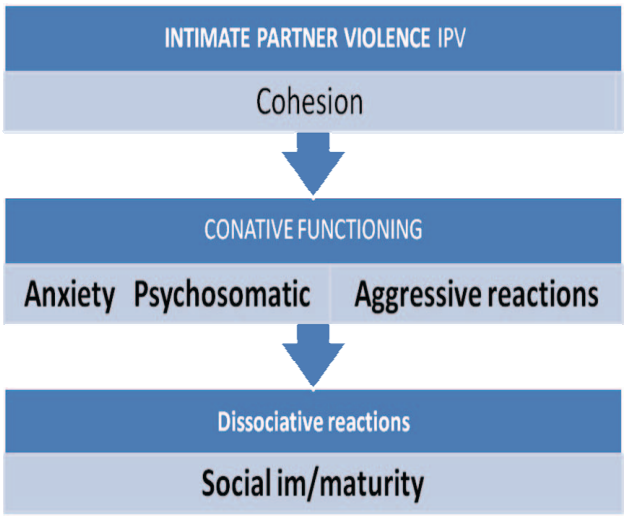


**Fig. 1 – The association between conative functioning of adolescents exposed to intimate partner violence and family dimensions.**

## Discussion

The theoretical and conceptual basis of the study was a family system theory, which defined a family with IPV as a dysfunctional system, caused by the dysfunction of a subsystem, or a violent parent, in which a multiple damaged spouse differentiation led to the impossibility of developing differentiation in children growing up in such families [18, 19].

The data obtained in the study indicated that IPV and a conflict in a marital dyad caused the significant disorders in the family functioning by reducing the family dimensions of cohesion and adaptability. The emotional difficulties, constant conflicts and different forms of violence in the functioning of marital dyad caused reduced closeness, cohesion, unity and trust among all family sub-systems. The Dimension category of high cohesion was statistically significantly less established in the IPV families as opposed to the functional non-violence families. Changes in cohesion within the IPV families were also confirmed by the regressive linear analysis, which determined the greatest influence of intimate partner family violence on this dimension. In the functioning of family dimension of adaptability, realization of disciplining children, setting norms, habits and boundaries in conduct, there was found a statistically significant, yet slight difference between the IPV families and functional non-violence families. The category of developed adaptability was more pronounced in the families without violence as opposed to the IPV families. The disorders in the IPV family parents' capability to fulfil family roles, disrupted quality of the parent–child relationship, and not being interested in the functioning of children were all associated with psychosocial adjustment and development of internalized and externalized symptoms in the adolescents coming from such families [20, 21].

Marital dyad imbalance was associated with the disorders of conative functioning of adolescents exposed to IPV. Adolescents adjusted to emotional, physical and sexual IPV through increased defence, attack and internal organ reactions [4, 22].

According to the Cybernetic model of personality dimensions, six hierarchical conative personality dimensions, cognitive and motoric system regulated the whole psychological functioning of the personality. The disorders of lower regulation functions: organic, defence and attack reaction regulator cause impaired functioning of higher systems for the coordinating and integrating regulation functions [17].

Conative functioning of adolescents growing up in families with IPV and exposed to IPV was significantly different in hierarchically lower and higher dimensions: defence regulator, attack, organic reactions, and systems for coordinating and integrating regulation functions, except for the hierarchically lowest activity regulator. Although the significant differences in the functioning of activity regulator was not found in the study did not find, the hierarchically lowest located system which regulates the energy level of all other systems, including cognitive and motoric processors, the category of pathological functioning of this dimension was more pronounced with the adolescents exposed to IPV from an early age, as opposed to the adolescents coming from the functional non-violence families. Pathological functioning of the activity regulator led to abulic, depressive and hypomanic reactions [2, 4, 8]. Given that the activity level determined the speed of information flow within the central nervous system, those disorders affected the efficiency of cognitive and motoric processes. The adolescents exposed to IPV experienced mental health consequences: psychological, social and academic difficulties in accomplishing school assignments [23].

The family cohesion disorders, such as self-control problems, were associated with the statistically significant differences in the functioning of hierarchically higher conative dimensions: defence/anxiety regulators, attack/aggressive and organic/psychosomatic reactions of adolescents exposed to IPV, as opposed to the adolescents coming from the functional non-violence families [24]. The adolescents growing up in the families with IPV and exposed to IPV showed a significant disordered functioning of the organic function regulator (χ), responsible for organic function centres, as opposed to the adolescents coming from the functional non-violence families. The category of pathological functioning of the organic function regulator was more pronounced in the adolescents growing up in the families with IPV and exposed to IPV. With approximately 10% of adolescents exposed to IPV, the disorders of this regulator could cause the functional disorders of the basic organic systems (cardiovascular, respiratory, urogenital, sensory, motoric), and control of biological processes, which secondarily formed a hypochondriac reaction system and the onset of psychosomatic illnesses [9, 13, 25].

The adolescents exposed to IPV had significantly more pronounced defence reactions, anxiety and trauma symptoms, as opposed to the adolescents coming from the functional non-violence families [5]. The category of pathological functioning of this conative dimension was found with almost one quarter of adolescents exposed to IPV. The disorders of regulator functioning of defence reactions (alpha) led to anxiety symptoms and formed the basis of pathological reactions such as: phobias, obsessive-compulsive disorders, sensory and emotional hypersensitivity [8]. Dysfunction of the regulator dimension of defence reactions coupled with activity regulator dysfunction formed the basis of possible future psychopathology, depression modalities and psychasthenia [26]. Along with the dysfunctions of the system for coordinating regulation functions (dissociative reactions), the defence regulator dysfunction caused more serious depressive, obsessive and compulsive reactions, while neuroticism was a consequence of defence and organic reaction regulator dysfunction [9, 27].

The significant disorders in the functioning of the superior system of coordinating regulation functions (delta), as opposed to the adolescents coming from the functional non-violence families.

Pathological functioning of the system for coordinating regulation functions was more pronounced with the adolescents growing up in and exposed to IPV. The pathological values Dysfunctional communication in IPV was characterized, among other things, by low levels of verbal expressiveness and emotional responsiveness, low tolerance to criticism and its interpretation as a threat, or intimidation, and consequently, increased anxiety and subsequent escalation of an argument into violence [28].

The attack regulator reactions (sigma), or aggressive reactions were significantly more pronounced with the adolescents growing up in the families with IPV and exposed to IPV, as opposed to the adolescents coming from the functional non-violence families. Because of the energy potential necessary for the aggression to be carried out, a model represented a significant relationship between the attack regulator and activity regulator. The attack reaction regulator dysfunctions were manifested by the variously modelled aggressive reactions and weak control of immediate impulses. The conative dimension of pathological values of aggressive reactions were found with one fifth of adolescents exposed to IPV. In the unfavourable development, this percentage of adolescents' increased aggressive reactions might develop various externalized symptoms: impulsiveness, destructiveness, conduct disorders, delinquency [5, 29]. The aggressive reactions of adolescents exposed to IPV were associated with the maternal mental health, lack of family warmth, and child maltreatment. Witnessed and experienced intimate partner physical violence, maltreatment and abuse in the family relationships caused the development of aggression, aggression perpetration in the future and a risk of trans-generational violence transfer to the adolescents [30, 31]. Dysfunction of lower conative regulators with the adolescents growing up in the families with IPV and exposed to IPV, such as psychosomatic, anxiety and aggressive reactions, was associated with of the system for coordinating regulation functions caused disorganization and dissociation of cognitive and conative processes and motoric function disorders. The schizoid, paranoid and manic symptoms were the direct products of this system disorder. The more serious disorders of system for coordinating regulation functions produced the secondary disorders of all the functionally subordinated systems: severe forms of sensory and motoric conversions, some forms of inhibitory conversions and fixed phobias, obsessions and compulsions [9, 22, 32].

The disorders of hierarchically lower conative dimensions with the adolescents exposed to IPV led to the significant differences in the functioning of the highest system for integrating regulation functions, etha, as opposed to the adolescents coming from the functional non-violence families. The system for integrating regulation functions (etha) integrated the conative changes in the form of psychological field structure, especially in the form of social field and changes in that field. Because of that, a set of programmes which determined the functions of this system was primarily formed during the process of upbringing, through conditioning and identification. Pathological functioning of the system for integrating the regulation functions were more pronounced with the adolescents exposed to IPV, as opposed to the adolescents coming from the functional non-violence families. Unfavourable development of pathological system for integration was associated with the social maladaptation, lower level of social maturity and externalized symptoms. The adolescent exposure to IPV, together with the parenting psychopathology and low self-regulation with the adolescents, influenced antisocial behaviour, alcohol addiction, aggression, hostile reactions and trans-generational violence transfer [20, 29, 33]. Dysfunctional familial relationships, family communication and interaction were often accompanied by suicidal behaviour.

The related scientific works stated that the protection factors for the adolescents exposed to IPV were found within family, school and social community. Family characteristics

were the most important ones when it came to the influence of violence on the appearance of the symptoms in adolescents: a) close family relationships and social support at school for internalized symptoms (e.g. anxiety, depression, post-traumatic stress disorder PTSD) and b) close family relationships for externalized symptoms (e.g., aggression, substance use) [34].

The limitation of this study and the possibility of new research was the fact that apart from the family dimensions of cohesion and adaptability, the associations among other forms of family functioning were not investigated (e.g., unemployment, material well-being, education level of the parents, psychopathology of the parents) and conative functioning of adolescents growing up in the families with IPV and exposed to IPV from early age.

The second limitation was that our results were based on self-reported information. Therefore, the offering could be connected with a high confidence in the capacity to describe own self.

This study has scientific relevance in clarifying the mechanisms linking family violence and cohesion with conative dysfunctioning as well as investigating the high risk of developing psychopathological behaviour.

**Conclusion**

This study suggested that growing up in a dysfunctional family with IPV, which damaged the multiple basic dimensions, communication and cohesion between the sub-systems and the exercise of parental roles, was associated with the cognitive, social, emotional, and behavioural problems, and significantly predicting psychopathological symptomatology of these adolescents as opposed to the adolescents from the functional non-violence families. The adolescents exposed to IPV showed the significantly higher pathological values for the following dimensions: psychosomatic, anxiety, aggressive, dissociative and social maladjustment reactions. Growing up in a dysfunctional violence family was accompanied by the continuous exposure to negative, stressful and traumatic experiences, which in turn was associated with intensified reactions of attack and defence, but also with weak potential for integrative functions. The data illustrates the need for introducing some preventive and psychotherapeutic measures in working with the adolescents exposed to IPV. The preventive measures for stopping the development of psychopathological internalized and externalized symptoms represented preservation, or restoration of close family relationships and social support at school.

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Received on May 14, 2017.
Revised on May 14, 2017.
Accepted on January 09, 2018.
Online First January, 2018.

ORIGINAL ARTICLE

UDC: 616.316-006-076
https://doi.org/10.2298/VSP170320001O

# The determination of specificity, sensitivity and accuracy of core needle biopsy in the diagnosis of parotid and submandibular salivary glands tumors

## Određivanje specifičnosti, senzitivnosti i tačnosti biopsije šupljom iglom u dijagnostikovanju tumora doušne i podvilične pljuvačne žlezde

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**Abstract**

**Background/Aim.** The diagnosis of tumors of salivary glands relies heavily on radiological examination and biopsy of pathological tissue. The aim of this study was to investigate the sensitivity, specificity and accuracy of core needle biopsy in diagnosis of tumors of parotid and submandibular glands. **Methods.** This study was designed as a cross-sectional clinical trial performed between May 2008 and May 2015 at the Department of Otorhinolaryngology and Maxillofacial Surgery, Clinical Center Zemun, Belgrade, Serbia. The examinations included 200 patients among which 100 were diagnosed with tumors of parotid salivary glands and 100 with tumors of submandibular salivary glands. The core needle biopsy was undertaken in all cases where tumor was smaller than 2 cm, far from blood vessels and far from the deep layer of parotid gland. The histopathological analysis was performed to identify histological type of the lesion. Upon performing the surgical procedure and consequently the tumor tissue extirpation, tissue samples obtained were investigated for the definitive diagnosis. **Results.** The sensitivity of the procedure was 90.9% for parotid salivary gland and 74% for submandibular salivary gland, the specificity was 95.9% for parotid salivary gland and 93% for submandibular salivary gland and the accuracy was 94.7% for parotid salivary gland and 87% for submandibular salivary gland. Based on the histopathological findings of the salivary glands obtained using core needle biopsy of the tumor tissue, it was possible to differentiate between malignant and benign lesions. **Conclusion.** Current investigation points to the advantages and efficiency of core needle biopsy in diagnosis of tumors of parotid and submandibular salivary glands.

**Key words:**
**biopsy, fine-needle; biopsy, needle; diagnosis; histological techniques; parotid neoplasms; sensitivity and specificity; submandibular gland neoplasms.**

**Apstrakt**

**Uvod/Cilj.** Dijagnoza tumora pljuvačnih žlezda se pretežno zasniva na radiološkom ispitivanju i histološkoj analizi patološke mase dobijene biopsijom. Cilj rada bio je da se ispitaju specifičnost, senzitivnost i tačnost biopsije šupljom iglom u dijagnozi bolesnika sa oboljenjima parotidne i submandibularne pljuvačne žlezde. **Metode.** Istraživanje je sprovedeno kao studija preseka u Odeljenju za otorinolaringologiju i maksilofacijalnu hirurgiju Kliničkog centra Zemun, Beograd, u periodu od maja 2008. do maja 2016. godine Studijom je bilo obuhvaćeno 200 bolesnika od kojih je kod 100 bio dijagnostikovan tumor u doušnoj pljuvačnoj žlezdi a kod 100 tumor u podviličnoj pljuvačnoj žlezdi. Biopsija šupljom iglom je bila sprovedena kod svih bolesnika kod kojih je tumor bio manji od 2 centimetara, udaljen od krvnih sudova i udaljen od dubokog režnja doušne pljuvačne žlezde. Posle toga je histološkim nalazom utvrđivan tip promene. Posle sprovođenja hirurške intervencije i potpunog uklanjanja obolelog tkiva, uzorak je ispitan za uspostavljanje konačne dijagnoze. **Rezultati.** Senzitivnost postupka iznosila je 90,9% za doušnu pljuvačnu žlezdu i 74% za podviličnu pljuvačnu žlezdu; specifičnost je bila 95,9% za doušnu pljuvačnu žlezdu; 93% za podviličnu pljuvačnu žlezdu i preciznost je bila 94,7% za doušnu pljuvačnu žlezdu i 87% za podviličnu pljuvačnu žlezdu. Na osnovu histopatološkog

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nalaza biopsije šupljom iglom bilo je moguće diferencirati maligne od benignih lezija. **Zaključak.** Sprovedeno istraživanje ukazuje na prednost i efikasnost biopsije šupljom iglom u dijagnozi tumefakata doušne i podvilične pljuvačne žlezde.

**Ključne reči:**
**biopsija tankom iglom; biopsija iglom; dijagnoza; histološke tehnike; parotidne žlezde, neoplazme; senzitivnost i specifičnost; submandibularne žlezde, neoplazme.**

## Introduction

The pathology of parotid and submandibular salivary glands tumors includes variety of inflammatory lesions as well as benign and malignant tumors [1–3]. Notably, the treatment is heavily dependent on the results of the histopathological outcome [4]. Numerous studies have shown that 70% of parotid tumors belongs to neoplastic lesions and among those 75%–80% are benign which consequently demand to exclude nonneoplastic lesions to avoid unnecessary interventions [5]. Precisely, it is required to set a correct diagnosis and exclude congenital, granulomatous and inflammatory ethiological factors leading to the gland enlargement [6]. The proper diagnosis relies heavily on the clinical examination, radiological procedures using ultrasound or magnetic resonance imaging and laboratory findings, but for the definitive diagnosis, biopsy followed by the histopathological analysis is necessitated [7, 8]. It allows the discrimination between the neoplastic lesions that require surgical intervention and nonneoplastic lesions that may be treated conservative [8].

Among currently available noninvasive biopsy techniques, a fine needle aspiration cytology (FNAC) is the most commonly used for the diagnosis of tumors of salivary glands [9]. Based on the extensive work already reported on FNAC, the average sensitivity and specificity for FNAC are 80% and 90%, respectively. Novel literature findings report the sensitivity of FNAC between 64% and 90% and specificity between 86% and 100% [10, 11]. One of the drawbacks of this technique is its low sensitivity that comes as a consequence of difficulties in diagnosis of low differentiated carcinomas only by cellular morphology. Other downsides of this methodology are the high level of false negative findings and unreliable accuracy for different malignity differentiation. Yet, this technique is often followed by the cytology-fluorimetry, immunochistochemistry or extirpation biopsy for the definitive diagnosis. In addition, the preciseness of FNAC is dependent on the institution where the procedure is performed, experience of the operator, ultrasound guiding, the presence of cytologist in the institution and accessibility of the methods such as flow cytometry which is sometimes required for additional analysis. Therefore, relatively high sensitivity and specificity are true only for institution with the standardized procedures for FNAC [11–13].

Recent developments in pathology of salivary glands introduced a core needle biopsy (CNB) as diagnostic tool with its increased specificity and sensitivity and decreased number of false negative results in comparison to other biopsy techniques [14]. The advantages of CNB in comparison to FNAC include the possibility for routine histopathological analysis, differentiating between *in situ* and invasive lesions and excluding the possibilities for false benign lesion diagnosis. The additional advantages come as a consequence of avoiding the general anesthetics use, shorter time required for hospitalization and faster return of patients to usual activities [15, 16].

The investigators found an abundance of promising evidence for the suitability of CNB in different tumor diagnosis. However, a careful literature overview does not seem to reveal a vast amount of data regarding its applications in tumors of parotid and especially submandibular region of head and neck. Having in mind all unknown aspects of CNB in tumors diagnosis in the orofacial region the specific aim of this study was to determine the specificity, sensitivity and accuracy of CNB for the diagnosis of the parotid and submandibular region pathology.

## Methods

### *Patients*

The current study was performed as a cross-sectional clinical trial between May 2008 and May 2015 at the Department for Otorinolaringology and Maxillofacial Surgery of the Clinical Centre Zemun and included 200 patients. In 100 patients, the biopsies were performed in the parotid salivary gland and in 100 cases in submandibular salivary gland. In all patients, the clinical examination or ultrasound diagnosis confirmed the presence of tumors in parotid or submandibular salivary glands. The criteria for involvement in the study were as follows: age between 18 and 80 years and clinically and radiologically verified enlargement of parotid and submandibular salivary glands. All patients gave the informed consent for participation in the examinations. The criteria for the exclusion from the study were the patients who did not came back for the control examination. The investigation was performed after obtaining the Institutional Ethics Committee Approval from the Clinical Centre Zemun – Belgrade.

### *Computed tomography imaging*

The parotid and sumbandibular salivary glands lesions in the investigated patients were imaged by using computed tomography imaging (CT) (MSCT, Toshiba) with the axial cross sections thickness measuring 0.5 × 0.3 mm and the width of the reconstructive interval of 0.2 mm. The CT scanning was performed in the native, arterial and venous phasis. Contrast was administered intravenously (70 mL) and the imaging was acquired after 65 s in order to obtain an optimum visualization of blood vessels of the neck.

*Core needle biopsy*

The CNB was performed by the same operator using the 18 gauge easy core biopsy device. The CNB was done after administration of local anesthetics (Xylocaine 1%) given under the skin by an insulin needle and minimally tissue damage. The knife No. 11 was used for incision and placements of needle pistol. After the needle administration, the tissue specimen was inserted into canila. The procedure was repeated twice. The specimens were stored in formalin solution and sent for the histopathological verification. In all cases where the intervention was performed, the extirpation biopsy was undertaken and sent to definitive histopathological diagnosis.

*Histopathological analysis*

The received specimens from the CNB were immersed overnight in 37% formalin solution for fixation, and processed with the standard methods for the histopathological analysis. All of the slides were stained with hematoxylin eosin (HE) method. In cases of diffuse large cell B lymphoma, or small cell lymphocytic lymphoma (diagnosis was established later on immunochistochemistry) only a slide description was made. The diagnosis of squamocellular carcinoma was readily made by using the slides from core biopsies.

*Statistical analysis*

The normality of the data distribution was evaluated by using the Kolmogorov-Smirnov test. The corresponding results were tabulated as true positive, true negative, false positive and false negative. Subsequently, the sensitivity, specificity and accuracy of CNB were calculated. The differences in the accuracy, specificity and sensitivity between the parotid and submandibular groups were compared by the $\chi^2$ test. Tumor subtyping was performed in order to demonstrate the distribution of histopatological findings and to identify the most common histologically known cases with the false positive or false negative outcomes. A *p*-value less than 0.05 was considered a statistically significant.

## Results

A total of 200 specimens from the parotid and submandibular gland were examined, among which 100 were from the parotid gland and 100 from the submandibular gland. Initially, in a preoperative phase, the salivary gland tissue was obtained by using the CNB, and the histopathological examination was performed. Thereafter, the final histopathological diagnosis was established by the examination of the salivary gland tissue obtained by the extirpation biopsies during the definitive surgical procedure. The patients with parotid tumors were divided into the groups of benign lesions (n = 73), or malignant (n = 22) conditions based on the final diagnosis. In the submandibular group, the same was undertaken for the benign (n = 61) and malignant (n = 31) cases. Five inadequate specimens in the parotid and 8 in the submandibular group were found. The accuracy, specificity and sensitivity of CBN are shown in table 1. There was no statistical significant difference in specificity finding between the parotid and the submandibular group, while the significantly higher sensitivity and accuracy were found in the parotid group ($p < 0.05$) (Table 1). The unsatisfactory tumor diagnosis tended to be higher in the submandibular salivary glands group (Table 2).

**Table 1**

**Comparison of diagnostic accuracy values of core needle biopsy in tumors of parotid and submandibular salivary glands**

| Characteristic | Specimens | |
|---|---|---|
| | parotid (n = 100) | submandibular (n = 100) |
| Specimens (n) | | |
| adequate | 95 | 92 |
| inadequate | 5 | 8 |
| false negative malignant | 2 | 8 |
| true positive malignant | 20 | 23 |
| true negative benign | 70 | 57 |
| false positive benign | 3 | 4 |
| Sensitivity (%) | 90.9 | 74 * |
| Specificity (%) | 95.9 | 93 |
| Accuracy (%) | 94.7 | 87 * |

**Statistically significant in comparison to the results obtained for parotid gland.**

The CT images of tumors in the parotid and submandibular region are shown in Figure 1. Figures 2 and 3 reveal the histopathological findings in some of the investigated cases.

## Discussion

The presence of tumors in parotid or submandibular salivary glands is a huge diagnostic and therapeutic challenge. Nonneoplastic, benign neoplasms or malignancies may cause such tumors [1, 17]. To this purpose, the CNB was used in the current investigation to obtain the representative specimens of tumor tissue and evaluate the possibility to differentiate the tumor subtypes after the histopathological analysis. The obtained results consistently indicated that the CNB are nearing the point when it can be considered as the option of the first resort in the salivary gland diagnosis. Methodology used may be considered representative and highly complementary with procedures widely accepted in clinics.

In the opinion of Kraft et al. [10], the evaluation of novel diagnostic techniques like the CNB should be performed by comparing the results obtained after the CNB and histolological findings after the extirpation biopsy. This methodological approach was followed in the current investigation. In line with the results of this study, the relevant literature data confirm the suitability of CNB for the salivary glands tumors diagnosis.

**Table 2**
**Unsatisfactory rates of core needle biopsy (CNB) in the salivary glands tumors according to the histological diagnosis**

| Neoplasms by histologic type | Unsatisfactory rates of CNB | |
|---|---|---|
| | parotid salivary gland | submandibular salivary gland |
| Malignant | | |
| Carcinoma of salivary gland | | |
| adenocarcinoma | 0/2 | 1/5 |
| carcinoma of acinus cells | 1/2 | 0/4 |
| cystadenocarcinoma | 0/1 | 0/1 |
| mucoepidermoid carcinoma | 0/1 | 2/2 |
| Lymphoma | | |
| Hodgkin | 0/5 | 1/4 |
| non Hodgkin | 0/3 | 1/4 |
| Metastasis in lymph nodes | | |
| squamous cell carcinoma | 1/5 | 1/6 |
| adenocarcinoma of prostate | 0/1 | 1/1 |
| melanoma | 0/2 | 1/3 |
| undifferential carcinoma of nasothroath (UCNT) | 0/0 | 0/1 |
| Subtotal | 2/22 | 8/31 |
| Benign | | |
| Adenoma | | |
| pleomorphic adenoma | 3/53 | 3/33 |
| adenoma of basal cells | 0/2 | 0/5 |
| oncocytoma | 0/2 | 0/5 |
| lymphadenoma | 0/4 | 1/16 |
| basal cells adenoma | 0/7 | 0/1 |
| lypoms | 0/5 | 0/1 |
| Subtotal | 3/73 | 4/61 |
| Total | 5/95 | 12/92 |

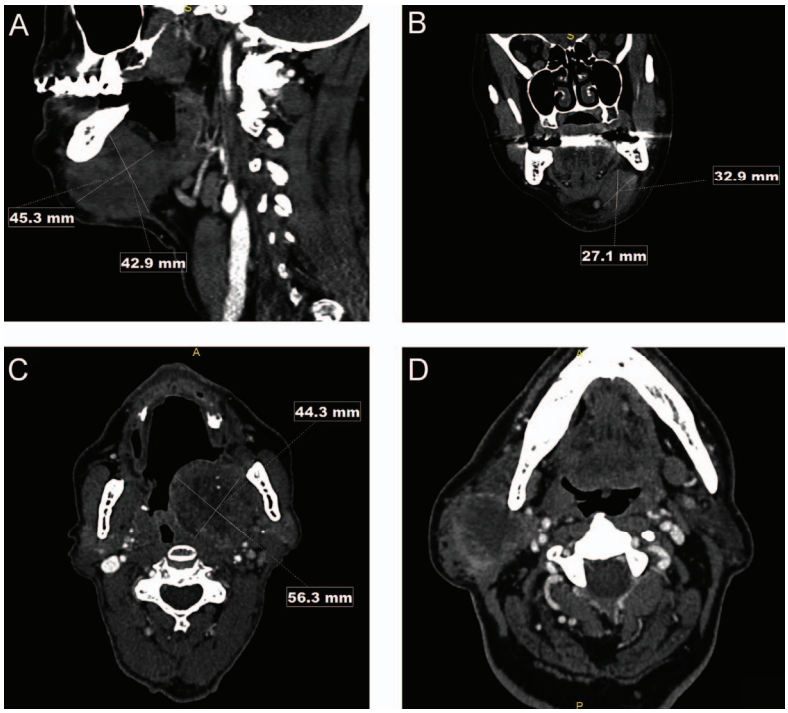


**Fig. 1 – Computed tomography imaging of tumors lesions in: A, B) submandibular and C, D) parotid salivary gland. A) Coronal cross section of tumor lesion in the left submandibular salivary gland is characterized as a soft tissue lesion measuring 45.3 mm × 42.9 mm; B) The sagittal cross section of tumor lesion in the left submandibular salivary gland measuring 32.9 mm × 27.1 mm; C) The saggital cross section of tumor lession in the right parotid salivary gland measuring 44.3 mm × 56.3 mm with invasion into the deep lobe of salivary gland; D) The tumor lession in the superficial part of the left parotid salivary gland (hematoxylin eosin staining, 200×).**

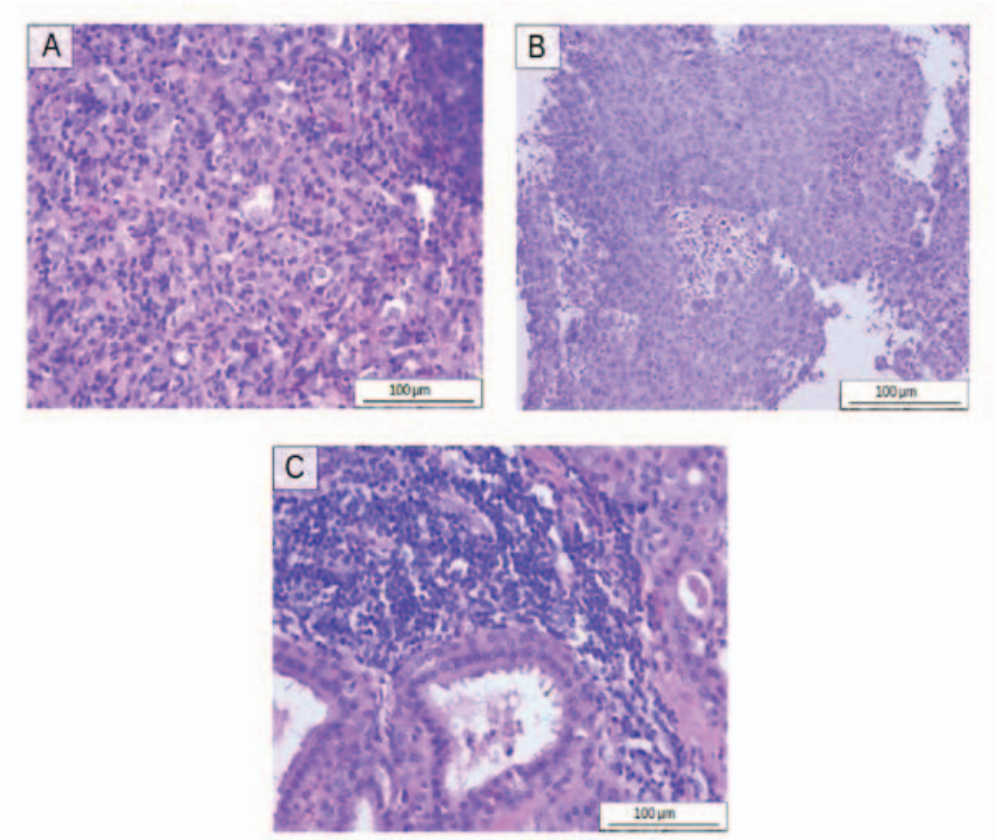


**Fig. 2 – Histology of tumors in the parotid salivary gland: A) A classic Hodgkin lymphoma can be recognized in the hematoxylin eosin core biopsy samples without difficulty by the presence of Reed Sternberg cell and its variants, even though their number can vary from case to case. In the most common type of Hodgkin disease, nodular sclerosis Hodgkin disease, numerous lacunar cells can be identified as large cells with polylobular nuclei and small nucleoli. Their acidophilic cytoplasm is affected by formalin fixation, so that the cells seem to lodge in an artificial lacuna. When such cells are seen in an admixture of inflammatory cells (lymphocytes, eosinophils), the diagnosis is usually straightforward, along with the adequate phenotype findings; B) Squamous cells carcinoma, especially when well differentiated, can be recognized as an epithelial neoplasm of large to medium cells with the ample eosinophilic cytoplasm, distinct cellular borders and large vesicular nuclei with the prominent nucleoli. Foci of keratinization can also be identified, as well as the mitotic figures; C) Warthin tumor, as a benign neoplasm, is a biphasic tumor, composed of a heavy lymphocytic infiltrate and an epithelial component of oncocytes. The glandular structures of this tumor are lined by two rows of large cells with intensive eosinophilic cytoplasm with basally oriented regular nuclei (hematoxylin eosin staining, 100×).**

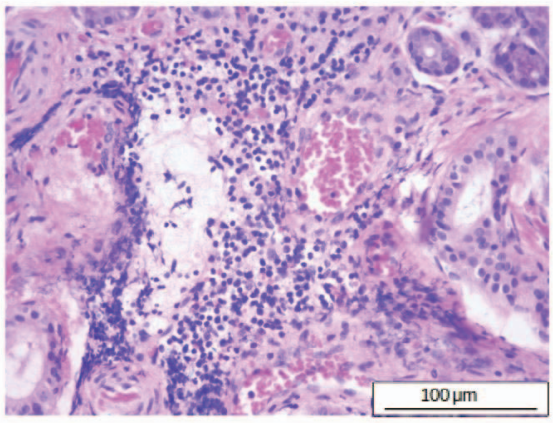


**Fig. 3 – Histology of tumor in the submandibular salivary gland. Tissue of mixed salivary glands is observed showing the signs of chronic sclerosing inflammation as well as dilatation of largerducts. Foci of microhemorrage are also noted (hematoxylin eosin staining, 200×).**

Howlett et al. [15] demonstrated the CNB sensitivity and specificity of 100% in the histopathological verification of neoplasms by evaluating 135 patients with tumors in the parotid region. In another study on the same topic, Screaton et al. [18] showed the sensitivity of 98%, specificity of 100% and preciseness of 99% of CNB in differentiating between the benign and malignant lympadenopathy. However, it is to note that only 23% of the patients were subjected to the open biopsy of lymphatic nodes of neck dissection while in the rest of the patients' diagnosis was confirmed by the clinical and laboratory findings. Also, in accordance to a meta-analysis performed by Schmidt et al. [11] , the average sensitivity and specificity of CNB was 80% and 97% respectively. Recent investigations showed that the sensitivity and specificity of ultrasound guided CNB is even higher, 99%–100% [5, 19].

Although scientific literature provides information on the diagnosis of tumors of using the CNB, FNAC and extirpation biopsy, the limited data is available to compare those procedures on the same patient. The investigators who compared the FNAC and CNB on the same tissue found an accuracy of CNB and FNAC to be 83% and 64%, respectively [20]. Also, an investigation of FNAC or CNB combined with a subsequent incision biopsy of the same bioptic mass showed the accuracy of incision biopsy was 100%, the accuracy of FNAC was 75% and the accuracy of CNB was 80.7%. However, in that study the histopathological diagnosis could be established correctly in only 33% of cases after FNAC and in 45% cases after the CNB. Therefore, the authors suggested the incision biopsy as a definitive diagnostic tool [10].

The CNB presents a safe, simple and efficient diagnostic procedure with preciseness higher than 97% in the diagnosis of parotid tumors [21]. It provides improved possibilities for obtaining the specimen of higher volume in comparison with FNAC decreasing the level of nonrepresentativity (in FNAC incidence is 8.1% and in the CNB 1.2%). This is of paramount importance in smaller medical centers where pathologists are not capable to analyze specimens immediately. In addition, the CNB preserves the histological architecture increasing the preciseness of the diagnosis and enabling the additional diagnostic procedures such as an immunohistochemically analysis [22]. The advantages of CNB are documented especially in cytological diagnosis of pleomorphic adenoma, Wartin's tumor and lymphoma that are traditionally hard to diagnose using only FNAC [23, 24]. Using the needles of different size leads to the same results, although the 18 gauge is considered the most appropriate. Several drawbacks of CNB observed during current investigation are related to the necessity for local anesthesia during the biopsy procedure, since the procedure is painful and morbidity is higher in comparison to FNAC if not performed properly [25]. Majority of studies have shown that, in the patients with tumors such as fibrosarcoma, pleomorphic skeletal sarcoma and hondroma, there is a high possibility for their dissemination into salivary glands tissue [16, 26].

An important advantage of CNB is the possibility to allow for the precise identification of pathological structures during the histopathological examination, while in FNAC, a histologist is capable only of detecting cells morphology. It is also significant to note that the increased speed of needle administration in the CNB technique decreased the necessity to move the patient during the procedure and consequent noncomfort [14, 27]. The patients who were previously subjected to radiological treatment due to the tumors in the region of neck are especially not indicated for FNAC owing to the presence of scar tissue and hence the CNB is preferable in such cases [27, 28]. In addition, the CNB provides the superior diferentiation between limphoid hyperplasia from lymphoma than FNAC as well as improved identification of different types and carcinoma grading [10].

From the clinical point of view, the difficulties arise when trying to establish whether the salivary tumor originates from the tissue of salivary gland, or it presents the metastasis of the tumors of other organs. It should be born in mind that in the recent years, due to the general increase in tumors incidence, the possibility for the metastasis to occur in the tissue of the salivary gland is also increased. The current widely accepted clinical and diagnostic approaches in salivary gland diagnosis do not provide possibility to precisely point to the exact origin of the tumor without biopsy [29, 30]. The present study confirms the increase in the metastatic tumors in the tissue of salivary glands. In this investigation, it is documented that higher malignancies could be observed in the submandibular salivary gland than in the parotid one, and consequently, the higher amount of metastatic cases were also observed within the submandibular salivary gland. Thus, the possibility to diagnose the tumors of other organs when performing the CNB of salivary glands is higher in the case of submandibular salivary glands. The discrimination between the primary tumor of the gland and metastasis is also important for the treatment of the metastatic tumors because in such case the most important issue is to determine the primary tumor location. The CNB is of paramount importance for metastasis of primary tumors as a simple and reliable technique that can be performed in the outpatient conditions and have a significant influence in preventive diagnostic procedures, having the benefit for both diagnostics and the therapy of patients. For instance, it was easily observed in the current investigation in the case of undifferential carcinoma of nasothroat (UCNT) that it could be diagnosed with difficulty in this primary stadium without the established CNB of submandibular salivary gland. In addition, in elderly patients where chronic inflammatory conditions resemble to the great extent the tumors, the CNB was a very reliable procedure to discriminate between those two pathologies.

Regarding the anatomical difference in the lymphatic nodes position in parotid and submandibular salivary gland, it should be noted that the lymphatic nodes in the parotid salivary glands are located within the gland tissue and in the case of submandibular salivary gland lymphatic nodes circumvent the submandibular gland in a close contact with the gland tissue - this complex of salivary gland-lymphatic nodes is considered to be a unit where the CNB was performed. In other words, metastases found in the lymphatic nodes surrounding the submandibular gland were considered in the present research as the gland metastasis.

The current investigation has some limitations. First, there are limited data in the literature related to the use of CNB for diagnosis of tumors of submandibular salivary glands which limits the possibilities for studies comparison. Yet, the real volume of biopsy tissue required for each case was in few instances smaller than initially estimated. Finally, it is not safe to assume that there were not the cases with dissemination of tumor tissue after biopsy because the patients were not monitored after the procedure long enough for such a conclusion (usually 20 years is required). However, all necessary measures were performed to minimize such a scenario including the excision of the needle pathway after excision, chemotherapy in the case of lymphoma presence and avoiding multiply injection into tumor tissue. From the pathological point of view, the limitation of CNB includes the diagnosis of Hodgkin and non-Hodgkin lymphomas – it was not possible to discriminate between those two tumors using the CNB technique. Although the literature data claim that the CNB is a suitable technique for such cases, these observations are mainly based on the radiological approaches that, in the opinion of the investigators of the current study, could not be easily performed in the routine clinical practice.

## Conclusion

The results obtained here for the tumors of parotid and submandibular region demonstrated the high sensitivity, specificity and accuracy of CNB as a diagnostic tool, and hence provide an additional verification for the suitability of the CNB in the clinical practice. Collectively, the data observed in the present study support the assumption that the CNB may be considered as a safe alternative to the open biopsy in the diagnosis of head and neck tumors. This technique is safe in all patients due to a short procedure and fewer complications in comparison to the excision biopsy. Altogether, the results of this study strongly recommend the use of CNB as a suitable and safe diagnostic tool in the pathology of parotid and submandibular salivary glands.

## Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 45005).

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Received on March 20, 2017.
Revised on December 17, 2017.
Accepted on January 08, 2018.
Online First January, 2018

ORIGINAL ARTICLE

UDC: 616.65-006-089
https://doi.org/10.2298/VSP170320076B

# Standard versus extended pelvic lymphadenectomy in the patients with clinically localized prostate cancer

## Standardna u odnosu na proširenu karličnu limfadenektomiju kod bolesnika sa klinički lokalizovanim karcinomom prostate

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**Abstract**

**Background/Aim.** Pelvic lymph node dissection (PLND) is the most accurate staging procedure in the diagnosis of lymph node involvement by prostate cancer. However, the therapeutic value of this procedure is still unclear. The objective of the study was to compare diagnostic and therapeutic values of extended and standard PLND as an adjunct of radical prostatectomy. **Methods.** The patients who underwent surgical treatment for clinically localized prostate cancer (n = 157) were enrolled in this open nonrandomized prospective study. In the standard PLND (sPLND) group 109 patients were enrolled while the extended PLND (ePLND) group involved 48 patients. Both groups were compared regarding age, prostate-specific antigen (PSA) level, a percentage of positive biopsies, preoperative and postoperative Gleason score, number of retrieved and positive lymph nodes, duration of surgery, blood loss, amount of lymphorrhea and biochemical recurrence-free survival. **Results.** The average number of retrieved lymph nodes was 17.27 and 24.46 in the sPLND and ePLND group, respectively ($p = 0.001$). The rate of positive lymph nodes was 9/109 (8.3%) and 8/48 (16.7%) in the sPLND and ePLND groups, respectively. Biochemical recurrence was noted in 38/109 (31.2%) and 7/48 (14.6%) patients in the sPLND and ePLND group, respectively ($p = 0.003$). **Conclusion.** Comparison of sPLND to ePLND led to the following conclusions: nodal yield was significantly higher in the ePLND group; the ePLND template was associated with a much higher rate of lymph node metastases; the biochemical recurrence-free survival rate was significantly more favorable in the ePLND group comparing to the sPLND group.

**Key words:**
**lymph node excision; pelvis; prostatic neoplasms; prostatectomy; surgical procedures, operative.**

**Apstrakt**

**Uvod/Cilj.** Karlična limfadenektomija predstavlja najpreciznu proceduru u dijagnostici metastaza karcinoma prostate u limfne čvorove. Međutim, njena terapijska vrednost još uvek nije jasna. Cilj ove studije je bio da uporedi dijagnostičku i terapijsku vrednost proširene i standardne karlične limfadenektomije u sklopu radikalne prostatektomije. **Metode.** Ukupno 157 bolesnika koji su hirurški lečeni radi klinički lokalizovanog raka prostate bili su uključeni u otvorenu nerandomizovanu prospektivnu studiju. U grupu standardne karlične limfadenektomije (sPLND) bilo je uključeno 109 bolesnika, a u grupu proširene karlične limfadenektomije (ePLND) 48 bolesnika. Obe grupe su bile upoređene prema starosti, koncentraciji prostate specifičnog antigena (PSA), procentu pozitivnih bioptata, Gleason skoru, broju odstranjenih i pozitivnih limfnih čvorova, trajanju operacije, procenjenoj količini gubitka krvi, količini limforeje i preživljavanju bez biohemijskog recidiva. **Rezultati.** Prosečan broj odstranjenih limfnih čvorova bio je 17,3 u sPLND grupi i 24,5 u ePLND grupi ($p = 0.001$). U sPLND grupi 9/109 (8,3%) bolesnika imalo je pozitivne limfne čvorove, a u ePLND grupi 8/48 (16,7%). Biohemijski recidiv ustanovljen je kod 31/109 (31,2%) bolesnika u sPLND grupi odnosno 7/48 (14,6%) bolesnika u ePLND grupi ($p = 0.003$). **Zaključak.** Upoređivanje sPLND i ePLND grupa dovelo je do sledećih zaključaka: proširenom karličnom limfadenektomijom se odstrani značajno više limfnih čvorova; prošenom karličnom limfadenektomijom dijagnostikuje se mnogo više metastaza u limfnim čvorovima; značajno je povoljnije preživljavanje bez biohemijskog recidiva u grupi proširene karlične limfadenektomije.

**Ključne reči:**
**limfadenektomija; karlica; prostata, neoplazme; prostatektomija; hirurgija, operativne procedure.**

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## Introduction

The incidence of lymph node metastases reported in the contemporary series of radical prostatectomies ranges between 2% and 57% [1, 2]. These differences may be a consequence of the extent of pelvic lymph node dissection (PLND) and inconsistent patient selection criteria. However, a lymph node involvement is an unfavorable prognostic factor for patients with prostate cancer.

Radical prostatectomy with, or without a PLND is a surgical procedure aimed to cure the patients with localized, or locally advanced prostate cancer. Despite the recent improvements of radiological imaging modalities, PLND is still the most accurate procedure for the diagnosis of lymph node metastases in the patients with prostate cancer [2]. Regardless of a surgical approach, either open or minimally invasive, PLND should provide an adequate surgical specimen of lymph nodes for the histopathological analysis. Retrieval of over 20 lymph nodes was considered as an adequate specimen for satisfying staging [3]. However, an autopsy study reported by Weingärtner et al. [4] showed the significant interpersonal variations in the pelvic lymph node count, even in the standard template of PLND. Therefore, a meticulous dissection in an anatomically defined template seems to be more important than the retrieval of certain number of lymph nodes. Unfortunately, there is still no consensus regarding the optimal PLND template. On the other hand, there are significant interpersonal variations among surgeons performing PLND with doubtful adherence to the proposed template [5]. Some authors suggested that the patients who underwent minimally invasive surgery had a lower yield comparing to those receiving an open surgical procedure [6].

The therapeutic value of extended PLND (ePLND) is controversial. However, several authors reported a long-term biochemical recurrence-free survival in the patients with the minimally invaded lymph nodes even without the androgen-deprivation therapy [7].

This prospective study was aimed to analyze the diagnostic and therapeutic value of two different templates of PLND used in our institution.

## Methods

During the period from January 2007 to December 2011, a total of 309 patients underwent the radical retropubic prostatectomy at the tertiary institution. The open nonrandomized prospective study was aimed to compare the diagnostic and therapeutic value of two templates of PLND that were used as an adjunct of radical prostatectomy.

The inclusion criteria were as follows: age up to 75 years, the preoperative prostate- specific antigen (PSA) level up to 25 ng/mL, 12-cores transrectal ultrasound (TRUS)-guided prostate biopsy with a complete histopathological report including the number of positive cores and primary and secondary Gleason grades, and completed clinical staging.

The non-inclusion criterion was the administration of neoadjuvant hormonal therapy.

The exclusion criterion was poor compliance with the follow-up schedule.

A total of 157 patients who fulfilled the inclusion criteria were included in the study. According to the template of performed PLND, the patients were enrolled into the standard PLND (sPLND) group (n = 109), or ePLND group (n = 48). The template of PLND was selected upon discussion between a surgeon and a patient.

Both groups were compared regarding age, the PSA level, a percentage of positive biopsies, the Gleason score, the number of retrieved and positive lymph nodes, the duration of surgery, blood loss and the amount of lymphorrhea and biochemical-free survival. Also, a total count of retrieved and lymph nodes as well as those bearing metastases within the sPLND and ePLND templates were analyzed in the ePLND group.

The sPLND template was bordered laterally by the genitofemoral nerve, distally with the inguinal ligament, proximally with the bifurcation of the common iliac artery, medially with the lateral bladder wall, and the internal iliac artery including obturator fossa with the completely skeletonized obturator nerve and the external iliac artery and vein (Figure 1a). The ePLND template was defined proximally with the common iliac vein, medially with perirectal and perivesical fat tissue, laterally with the genitofemoral nerve and lateral pelvic wall and distally with the inguinal ligament, with the completely skeletonized common iliac vein, the internal iliac artery and vein, the external iliac artery and vein as well as the obturator nerve (Figure 1b). The procedure was completed with the removal of the prostate within the prostatic capsule and seminal vesicles and creation of vesicourethral anastomosis over 18 Fr three-way Foley catheter leaving the drains bilaterally.

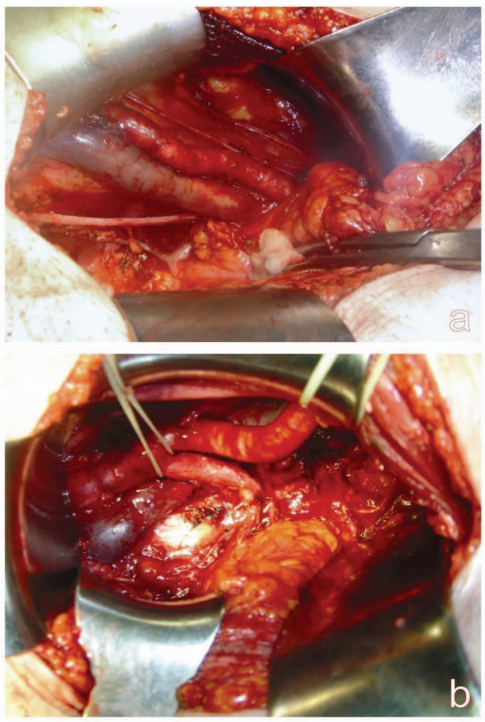


**Fig. 1 – a) Standard pelvic lymph node dissection (PLND) template; b) extended PLND template.**

Prostate cancer was confirmed by the histopathological examination of 12-core transrectal ultrasound-guided prostate biopsy specimen. The Gleason score was determined by different pathologists according to the International Society of Urological Pathology (ISUP) criteria [8]. A percentage of positive biopsies was calculated as the ratio of the number of positive biopsies/total number of biopsies.

All patients were staged by the digital rectal examination, computed tomography (CT) of abdomen and pelvis, and bone scan. All patients underwent surgery under the general anesthesia. A radical prostatectomy specimen was handled according to the recommendation by Montironi et al. [9]. Two dedicated uropathologists analyzed the surgical specimen for the lymph node count, lymph node metastases, extraprostatic extension, seminal vesicle invasion, status of surgical margins and Gleason score. Immunohistochemistry was not available during the observed period, and only the haematoxylin and eosin (H&E) staining was used.

The digital rectal examination and determination of PSA level were used for the routine follow-up at six weeks after surgery, every three months during the first year, and thereafter twice annually until July 2012. Biochemical recurrence was defined as the presence of two consecutive and rising PSA values above 0.2 ng/mL. Time to the biochemical recurrence was recorded prospectively during the outpatient visits.

The statistical analysis was performed using "Smart line agency" packet of statistical programs. The parametric data were analyzed using the Student's *t*-test and ANOVA. The categorical data were analyzed by using Pearson's $\chi^2$ test. Biochemical recurrence-free survivals were shown as the Kaplan–Meier estimates and overall group differences were evaluated by the log-rank statistics.

The study was conducted according to the principles of the Helsinki declaration and it was approved by the Ethics Committee of our Institution. An informed consent was obtained from all individual participants included in the study.

## Results

The patient demographics and biopsy characteristics of prostate cancer are shown in Table 1. The patients' age in both groups was similar and differences were not statistically significant ($p = 0.865$, Student's *t*-test). The mean PSA level was 10.30 ng/mL and 12.44 ng/mL in the sPLND group and the ePLND group, respectively. Although the mean values were close within the intermediate-risk group range, the difference was statistically significant ($p = 0.012$, Student's *t*-test). Also, the patients who received ePLND had a higher percentage of positive biopsies than those receiving sPLND, but the difference was not statistically significant. The distribution of biopsy Gleason scores was similar in both groups.

The variables that characterized surgery are shown in Table 2. The mean duration of surgery was 218.5 minutes and 204.5 minutes in the ePLND and sPLND group, respectively. The difference was statistically significant ($p = 0.043$, Student's *t*-test). The mean blood loss was significantly higher in sPLND than in ePLND ($p = 0.009$, Student's *t*-test). The average postoperative drainage was 1491.50 mL and 1158 mL in the ePLND and sPLND group respectively. However, the differences in the total amount and duration of postoperative drainage were not statistically significant.

**Table 1**
**Preoperative characteristics of the study population**

| Variable | sPLND | ePLND | *p* |
|---|---|---|---|
| Age (years)[a] | 65.14 ± 5.78 | 65.27 ± 6.02 | 0.865* |
| PSA level[a] (ng/mL) | 10.30 ± 5.08 | 12.44 ± 4.41 | 0.012* |
| Percent of positive biopsies[a] | 37.03 ± 24.35 | 43.68 ± 29.41 | 0.138* |
| Biopsy, Gleason score (n) | | | 0.364** |
| 4 and 5 | 38 | 9 | |
| 6 | 32 | 17 | |
| 7 | 35 | 19 | |
| 8 to 10 | 4 | 3 | |
| Total | 109 | 48 | |

**[a]results are given as mean ± standard deviation.**
**sPLND – standard pelvic lymph node dissection;**
**ePLNS – extended pelvic lymph node dissection;**
**PSA – prostate specific antigen;**
**n – number of patients; *Student's *t*-test; **$\chi^2$ test.**

The postoperative pathological staging and Gleason scores are shown in Table 3. The patients in the ePLND group had more commonly the locally advanced disease and lymph node metastases than those in the sPLND group. Although the lymph node metastases (pN1) were diagnosed more frequently following ePLND, this difference was not statistically significant ($p = 0.119$). Also, there was no association between the distribution of postoperative Gleason score in the investigated groups.

**Table 2**
**Surgery-related features in the investigated groups**

| Variable | sPLND mean ± SD | ePLND mean ± SD | *p* |
|---|---|---|---|
| Number of lymph nodes | 17.27 ± 5.66 | 24.46 ± 10.98 | 0.001* |
| Duration of surgery (min) | 204.5 ± 38.33 | 218.54 ± 43.45 | 0.043* |
| Average blood loss (mL) | 826.84 ± 549.07 | 590.96 ± 444.84 | 0.009* |
| Drainage (mL) | 1158.13 ± 1517.77 | 1491.50 ± 1570.77 | 0.081* |
| Drainage (days) | 12.89 ± 4.34 | 14.29 ± 5.48 | 0.084* |

**Abbreviation under Table 1.**
**SD – standard deviation; *Student's *t*-test.**

**Table 3**

**Distribution of pathological stages and the Gleason score in the investigated groups.**

| Parameters | sPLND n (%) | ePLND n (%) | *P* ($\chi^2$ test) |
|---|---|---|---|
| Pathological stage | | | 0.004 |
| pT0 | 3 (2.75) | 0 | |
| pT2 | 82 (75.23) | 30 (62.50) | |
| pT3a | 8 (7.34) | 9 (18.75) | |
| pT3b and 4a | 16 (14.68) | 9 (18.75) | |
| pN1 | 9 (8.25) | 8 (16.67) | 0.119 |
| Postoperative Gleason score | | | 0.065 |
| not available (pT0) | 3 (2.75) | 0 | |
| 4 and 5 | 20 (18.35) | 5 (10.42) | |
| 6 | 40 (36.70) | 13 (27.08) | |
| 7 | 36 (33.03) | 21 (43.75) | |
| 8–10 | 10 (9.17) | 9 (18.75) | |

**Abbreviation under Table 1.**
**n (%) – number (percentage) of patients.**

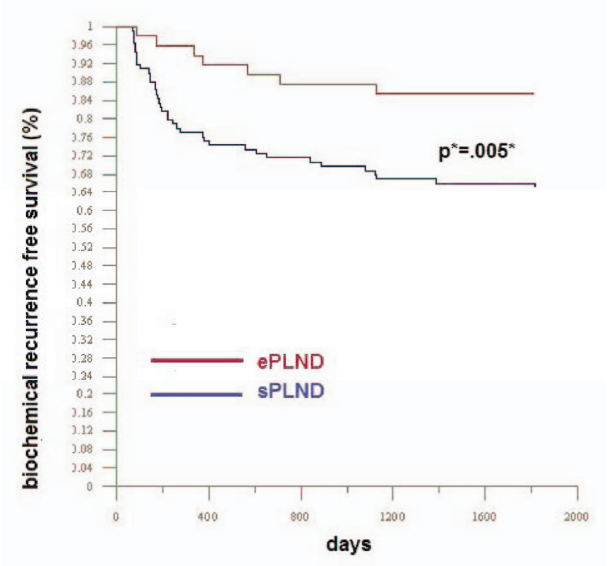


**Fig. 2 – Kaplan-Meier plots of biochemical recurrence-free survival in the sPLND and ePLND groups. Abbreviations under Table 1.**

There was a statistically significant difference in the lymph node yield among the sPLND and ePLND groups ($p = 0.003$, Student *t*-test). A total of 1,882 lymph nodes was removed in the sPLND group; the average number of lymph nodes was 17.27 ± 5.66 (range 8 to 34). The total of 1,174 lymph nodes was retrieved in the ePLND group; the mean number was 24.46 ± 10.98 (range 9 to 73). This nodal yield consisted of 861 and 313 lymph nodes removed within the sPLND template and hypogastric and presacral regions, respectively. The average number of retrieved nodes was 17.94 ±7.59 within the sPLND template and an additional 6.53 ± 4.35 in the hypogastric and presacral region.

In the sPLND group 9 (8.25%) patients were found to have the lymph node metastases. Eight (16.67%) patients in the ePLND group were diagnosed to have the nodal metastases. The lymph node metastases were found exclusively within the sPLND template in 2 (25%) patients. The lymph node metastases were detected exclusively in the hypogastric and presacral region in 3 (37.5%) patients. Another three patients had positive nodes within both templates. Therefore, increasing of nodal yield for 24.46% led to increased detection of lymph node involvement by 37.5%.

However, 40% of patients with sPLND group retrieved less than 15 lymph nodes, while 26.61% had 22 or more lymph nodes. Only 16% of surgical specimens contained less than 15 lymph nodes in the ePLND group, and 50% had 22 or more lymph nodes. There was also a statistically significant difference ($p = 0.006$; $\chi^2$ test).

The biochemical recurrence-free survival was more favorable in the ePLND group. Figure 2 represents the Kaplan-Meier plots for biochemical recurrence-free survival in both groups.

## Discussion

Radical prostatectomy with PLND is the treatment option for the patients with high-risk prostate cancer as well as a substantial proportion of those with intermediate-risk disease. Nowadays, the ePLND is recommended whenever a lymph node dissection has to be performed in these patients [10]. However, the limits of ePLND are still controversial. Currently, there are a few suggested templates of ePLND. The original, extended PLND template included a dissection of lymph nodes within the obturator fossa, external and internal iliac region. Recently, it was suggested that the presacral lymph nodes should be included in the ePLND template, too. The super-extended PLND means an additional dissection of lymph nodes in the common iliac region [11].

The clinicians dealing with the surgical treatment of prostate cancer are truly lacking a reliable radiological tool for the detection of positive lymph nodes. It is not expected that radiological imaging will be improved to the extent of detecting lymph node micrometastases in the near future. Therefore, we still need to adhere to meticulous PLND within extended templates. Generally, there are significant interpersonal and inter-institutional variations in the performance of this procedure. A surgeon seems to be the most important risk factor for a lymph node yield. Obviously, few surgeons who were performing radical prostatectomy in this study did not adhere to the recommended ePLND template.

Our data clearly showed that sPLND provides significantly fewer lymph nodes than ePLND. Also, this study showed that a substantial proportion of patients who received ePLND had a lower nodal yield than some patients receiving sPLND. This phenomenon can be explained by the inter-individual variations of pelvic lymph node count. Weingärtner et al. [4] analyzed a lymph node count on 30 cases in an autopsy study within the standard template only. They found significant interpersonal variations of lymph nodes count in the range from 8 to 56.

Although the rate of lymph node metastases was twice as higher in the ePLND group, the difference was not statistically significant. A lower percentage of diagnosed lymph node metastases in the ePLND group may be a result of restrictive inclusion criteria with the upper PSA level < 25 ng/mL.

The nodal yield of 20 lymph nodes was considered adequate for the reliable pathological staging [3]. Although the lymph node count is suitable for statistical analysis, it may represent a problem in clinical practice because a substantial proportion of patients receiving ePLND do not have 20 lymph nodes in their surgical specimen. In our opinion, there are five anatomical regions of pelvic lymph nodes: external iliac, obturator, hypogastric (internal iliac), presacral, and common iliac group. With an increasing number of these anatomical regions within the PLND template, there is also increasing the probability of accurate staging and complete dissection of involved nodes. Duration of surgery was increased significantly by more extensive lymph node dissection. Studer and Collette [12] reported that extended PLND increased the duration of surgery for 30 minutes approximately. The smaller difference was found in this study, probably because less experienced surgeons who required more time for prostate removal performeds PLND. It was also reflected in the amount of intraoperative blood loss. The amount of drainage was higher in the ePLND group, but this difference was not statistically significant. Capitanio et al. [13] reported a positive association between the amount of lymphorrhea with the number of removed lymph nodes and patients'age. The surgeons in both groups have used different techniques for lymph vessels control such as ligation, electrocautery, and harmonic scalpels. The influence of dissection techniques on the severity and duration of lymphorrhoea was not investigated in this study.

Lymph node metastases are an unfavorable prognostic factor in prostate cancer patients. However, many authors have reported a possible therapeutic role of PLND, particularly in patients with 1 or 2 positive nodes. Schumacher et al. [14] and Seiler et al. [15] reported that 20% of patients with one positive node have a chance to remain free of recurrence even without adjuvant hormonal treatment. Also, ePLND provided superior treatment outcome in terms of biochemical recurrence-free survival than sPLND even if the pN0 stage was confirmed. This treatment effect may be based on two facts: s PLND cannotremove positive nodes outside of the used template, and ePLND may remove more nodes with the unrecognized micrometastases. The patients who have experienced early biochemical recurrence after the radical prostatectomy with sPLND were diagnosed with the Gleason score ≥ 7, or PSA level > 10 ng/ml. Therefore, all patients with a high-risk prostate cancer (Gleason score ≥ 8, and/or clinical stage ≥ T3a, and/or PSA level >20 ng/mL) and a substantial proportion of patients with intermediate-risk disease (Gleason score 7, and/or clinical stage T2b, of T2c, and/or PSA level 10–20 ng/mL), particularly those with the primary Gleason grade 4 in the prostate biopsy, had a clear indication for PLND. It is recommended to perform an ePLND when it deemed necessary. The patients with low-risk prostate cancer (Gleason score < 7, PSA < 10 ng/mL, and clinical stage ≤ T2a) are not candidates for PLND [10, 16].

This study has certain limitations. Inapplicability of randomization is a potential limitation of the study design. Also, the heterogeneity of study groups may be another limitation of the survey. However, this problem is common in the majority of single-center studies. Further concerns are related to possible selection bias and the interpersonal variations in the experience and the expertise level in performance of PLND among different surgeons in the study.

## Conclusion

The extended pelvic lymph node dissection was clearly superior to the standard pelvic lymph node dissection in terms of nodal yield. Detection of metastatic lymph nodes was much higher following the extended pelvic lymph node dissection.

Biochemical recurrence-free survival was significantly less favorable following the standard pelvic lymph node dissection. However, the therapeutic value of extended pelvic lymph node dissection has to be confirmed in further investigations.

## Acknowledgments

The authors are thankful to Mr. Milan Dolga for statistical analysis of all collected data. We are also grateful to Mrs. Ivana Knezevic, MD, PhD, Faculty of Biology and Medicine, University of Lausanne, Epalinges, Switzerland for comments and suggestions for improving the manuscript. The authors are thankful to Mrs. Georgia Solaja for the improvement of language style of the manuscript.

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Received on March 20, 2017.
Revised on June 16, 2019.
Accepted on July 18, 2019.
Online First September, 2019.

GENERAL REVIEWS


UDC: 174:61]:350.6
https://doi.org/10.2298/VSP171026190P

# Mental capacity as medicolegal prerequisite for consent or refusal of medical treatment

## Sposobnost za rasuđivanje kao medikolegalni preduslov pristanka ili odbijanja medicinskog tretmana

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**Key words:**
**ethics, medical; informed consent; legislation; jurisprudence; mental health; physician-patient relation; therapeutics; risk assessment; serbia.**

**Ključne reči:**
**etika, medicinska; informisani pristanak; zakonodavstvo; pravna nauka; mentalno zdravlje; lekar-bolesnik odnosi; lečenje; rizik, procena; srbija.**

### Introduction

Occurrence and development of certain mental conditions does not necessarily place adults in such a position as to require legal protection. For a significant number of these patients their incapacity (or reduced capacity) to be rational and/or capable of making decisions has not been judicially ascertained.

However, their age, clinical symptoms, social context, sanitary or economic situation, place them in a vulnerable position. Such incapacity is not recognized by the existing system of legal protection [1], as their state is not permanent. However, this raises the question of legal validity of their cognition and willingness to conduct legal affairs pertaining to their property and assets. As they are positioned somewhere between capable and incapable [2], they must be given special protection only when their capacity to consent and/or to express willingness is diminished or nonexistent [2]. Unlike adults deprived of contractual capacity, who have been deprived of their rights in order to be protected, these people should be protected in such a way to be given more rights, although the boundaries between these two categories are very unclear [3].

These circumstances may, among other things, have certain repercussions when it comes to the patients' rights, according to the Act on Patient's Rights (APR) [4]. The problems become particularly apparent when a person admitted to a medical institution as a patient refuses the recommended medical treatment, yet shows diminished cognitive and conative capacity due to the present medical condition and its negative influence on patient's overall functioning. In such situations, the treating doctor should assess whether the patient is capable of consenting to treatment, that is, whether he/she has mental capacity. Consent to medical treatment does not qualify as a legal act [5] so it can not be equated with consent to contractual obligations and the assessment thereof. Accurate assessment of the patient's capacity in such circumstances is an important legal and ethical issue in medicine. Any inaccuracy in its assessment during medical treatment may have adverse effect or even fatal outcome. Such legally impermissible act or offence can render the doctor legally liable under misdemeanor, or civil law [5].

The issue is who may provide a required consent, if the patient is assessed as mentally incapacitated? It cannot always be the patient's legal representative, if he/she does not have one. Should the decision in this case be made by the family or the doctor? Legislation of the Republic of Serbia provides no answers to these questions. Even if such a person has, in accordance with APR (art. 16, section 5) appointed in advance an agent to provide consent, anticipating his/her future condition, the final decision (about any medical treatment) is always to be made by the doctor [6]. Doctor's definitive assessment of the patient's capacity to accept or refuse medical treatment is necessary, as it is a prerequisite for administering medical treatment. It is based on clinical assessment, and aims to protect patient's rights in each specific situation. These assessments are purely descriptive, but invariably involve normative issues.

On the grounds of the previous facts, we have determinated the main goals of this paper. It is to consider

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some legal and medical aspects of mental capacity assessment especially in the patients who refuse medical treatment. Also, we aim to suggest possible legislative improvements in the domain of patients' rights, with the focus on possible situations where the assessment is needed and which medical professionals should be in charge of.

**Consent to medical treatment and autonomy**

Before any medical treatment is administered, the patient must agree to it if he/she has legal capacity (APR Art 15 and 19). If the patient is deprived of contractual capacity, consent must be obtained from the patient's legal representative/person authorized by the law to represent patient and protect his/her rights. Furthermore, prior to making the decision about the treatment, a doctor is required to obtain the informed consent – to provide all the necessary information so that the patient can fully understand what he/she agrees to. Exceptionally, consent is not required in universally recognized cases such as emergencies when the patient's life is threatened, when such consent is assumed. According to the APR, these cases are defined as those when emergency treatment is administered to a patient who is not conscious, or who is not able to provide consent (art. 18, section 1). This suggests that mental incapacity is not expressly envisaged as an exception to consent to medical treatment, but as a condition to provide legal protection to such patients.

It is well-known that the right to consent to certain medical treatment usually implies the right to refuse it as well. In the common law countries, the right to consent to medical treatment is legally based in the right to self-determination [7], which is the individual right to make decisions about one's own life freely, following one's goals, as defined back in 1914 in the ruling of the US Supreme Court, Schloendorff v/Society of New York Hospital [8].

In continental law legal systems, this is based on the principle of inviolable right to physical and mental integrity. As a capacity to make autonomous decisions, it is also the prerequisite for the right to self-determination [9]. The principle of personal autonomy pertains to medicine as well, since the person who makes decisions about his/her body and health actually exercises the right to self-determination. A lack of mental capacity risks personal autonomy [10], especially with precarious medical treatments when a refusal of life-saving treatment can have fatal consequences. The mental capacity assessment should draw the line between the right to personal autonomy and obligation to provide legal protection [11]. This affirmation of the right to self-determination and personal autonomy, which became the basis of patient's right to consent to medical treatment, could not pass without conflict. Not only did it improve the patient's right, but it also emphasized the difference between medical and legal profession. Each of these two attempts to operate in the best interest of the third party – the patient [12].

Recently introduced legislative changes in Serbia have been heavily influenced by international law so as to promote the principle of personal autonomy within the concept of right to health protection, especially of persons deprived of contractual capacity [13]. The latter can receive medical treatment if their legal representative or statutory agent agrees to it (APR, art. 19, section 1). The medical professional in charge, however, must make it possible for the patient to be involved in the decision-making process to the extent his maturity and mental capacity allow. In other words, no individual deprived of contractual capacity is automatically and in every situation incapable of making decisions regarding his/her health nor is every individual with contractual capacity always capable of giving proper consent to medical treatment. However, in the latter case, due to their legal invisibility, this affirmation has bypassed those individuals who are not under legal protection, yet whose mental capacity may be diminished, or absent at a given moment in a given situation. Consequently, a patient's mental capacity is a prerequisite for consent to, or refusal of medical treatment. If a patient has mental capacity, medical treatment can be administered only if he/she consents to it. Otherwise, if the patient is mentally incapacitated, decisions about medical treatment can be made on other grounds.

**Legal assessment of mental capacity**

Mental capacity is primarily a legal category. In denoting mental illnesses, law applies its own terminology, which is broad enough to avoid frequent changes, as psychiatric terminology is very diverse and subject to numerous alterations [14]. In relation to medical treatment, mental capacity can be defined as the ability to understand the importance of agreeing to medical treatment and its potential consequences. It presupposes that the patient is conscious: the patient must understand presented facts and information and be able to weigh them rationally when making the decision [15]. There is assumption that all adults have mental capacity, the assumption that serves to protect their personal autonomy [16]. This assumption can be dismissed by a court decision, or medical assessment conducted while applying medical treatment. A person deprived of contractual capacity due to mental incapacity cannot give valid consent to medical treatment. Conversely, a consent given by a person with mental capacity is valid and mandatory. The problem is with previous temporary mental incapacity conditions that cannot be limited to mental illness diagnoses. Decisions on mental incapacity carries a variety of risks including a possible violation of the right to autonomy, integrity and personal dignity.

Assessment of capacity to consent to medical treatment is essentially legal in nature, and it is performed by physicians. It is based on the definition of 'mental capacity' which is, according to the APR, expressly required only when the patient refuses the suggested medical treatment. Namely, art. 17, section 1 stipulates that 'the patient with mental capacity has the right to refuse the suggested medical treatment, even when it saves or sustains his/her life.' Legislation of the Republic of Serbia does not provide a definition of mental capacity, except for minors above 15 in the APR, where it is defined as a child's capacity to understand the nature of his/her health condition, the purpose of the proposed medical treatment, the risks and consequences involved if the treat-

ment is applied or not applied, as well as the capacity to weigh the presented information while making the decision (Art 2).

Mental capacity is a factual state, relative and subjective. Its assessment is not conducted in a general manner, as it used to be, but functionally [10]. The functional approach to capacity to consent to medical treatment starts from the 'natural contractual capacity' of the person in question, regardless of his/her legal status [17]. It is indisputable that a person can be incapable of giving consent to medical treatment, yet capable of making all other decisions. For instance, the cognitive capacity of an Alzheimer patient may vary seriously from one day to another. Such functional and individual approach promotes respect for personal autonomy, where actual capacity is preserved. This is the starting point of the Convention on the Rights of Persons with Disabilities, according to which all protective measures must be individual and adapted to a person's disability [1]. It focuses on functional parameters of a person's state when making a concrete decision – the patient's ability to make a clear and valid choice in the given situation [10].

The assessment of the capacity to decide whether a medical professional should administer medical treatment can be performed only on patients who voluntarily consented to medical treatment, as his/her mental state can vary. Medical treatment can be imposed on a patient who is hospitalized against his/her will in cases listed in the Act on Protection of Persons with Mental Disorders (art. 21) [4]. The patient who voluntarily agreed to hospitalization has the right to accept or refuse medical treatment, although this sometimes seems to be a rather theoretical possibility, especially in situations when a doctor has doubts about the patient's mental state. This primarily pertains to cases where the patient refuses medical treatment which is urgent. In practice, however, urgent medical treatment is understood in quite general terms so as to limit the obligation to assess the patient's mental capacity or ask for legal representative's consent. Therefore, in terms of assessment it seems that it makes no difference whether the patient was admitted to hospital voluntarily or not.

The wording in Art 18. sec. 1 of APR pertaining to urgent medical treatment suggests that it can serve as an excuse to medical professionals to relieve them of responsibility or ethical duty, rather than as an exception to obligatory consent and the mental capacity assessment. One can even ask whether this exception can cover all the situations where medical treatment is given without consent [18]. The answer primarily depends on how urgent medical treatment is defined. Urgent medical treatment is not defined in legislation. To interpret it, it may be useful to resort to analogy with urgent medical assistance, regulated in art. 53 of the Law on Health Insurance [19] as 'immediate medical assistance provided so as to avoid putting the insured person in danger to life, that is to avoid irreversible or serious deterioration of or damage to his health or death.' In legal theory, it is defined as surgical or psychiatric treatment where diagnosing and treatment cannot be postponed due to the symptoms, and the emphasis is placed on its restrictive interpretation [20].

According to a Swiss medical case study [21], the capacity assessment should take into consideration four basic elements. Firstly, the mental capacity assessment is a case-specific. Secondly, the mental capacity is either present or absent. In practice, this implies that it is necessary to determine the sufficient capacity level in order to establish whether it is present or not in the given case, which means that it is gradable. The evaluation of the sufficient capacity level depends on the severity of the suggested medical treatment – the potential risk to patient's life and health. Thirdly, the capacity is a condition: as such it must be assessed during the decision-making process, rather than in relation to its outcome. This means that a patient can have mental capacity when making the 'wrong choice' from the assessor's point of view, or not have it when making the 'right choice' [21]. Fourthly, in any case, the results of the assessment must be documented.

Even when it is established that the patient suffers from mental disorder or intellectual development disability, toxicomania, or psychopathological symptoms, it does not imply in itself that the person lacks mental capacity. A patient can be schizophrenic, severely depressed, demented and so on, and yet possess mental capacity to make certain decisions. The fundamental question is to which extent these conditions, affecting psychological capacity, are deemed decisive when assessing mental capacity. Although there are the confirmed statistical correlations between the foregoing conditions and psychological capacity, there are a number situations where it is possible to show that a certain number of such patients are capable of making decisions regarding administration of medical treatments. Furthermore, the same diagnostic group comprises a large number of heterogeneous psychological states [22].

## Medical assessment of mental capacity in case of refusal of medical treatment

### *Medical aspect of mental capacity*

The medical assessment in these situations focuses on evaluating mental capacity in relation to the recommended medical treatment. Although different bodies of law link mental capacity to lower or higher level of psychological capacity [23], in general it can be claimed that the mental capacity encompasses a person's ability to understand the meaning of his/her actions: to understand the real, natural and legal importance of his/her acts. This capacity predominantly comes from the preserved intellectual (cognitive) mental functions and the capacity to govern one's actions: to adequately direct and take actions, and make decisions based on the preserved motivational processes – that there are no volitional deficiencies when certain actions are taken or not taken.

When applied to refusal of treatment, this means: the person has the capacity to understand the nature of the treatment and the manner of its administration, the importance of the treatment for the patient's health, the health condition he/she is in and its relation to the recommended treatment, as well as the potential negative consequences if the treatment is not given,

the risks involved if the treatment is refused and its possible side effects if accepted and that the patient is capable of making the decision to consent to or refuse the recommended treatment independently and rationally, weighing pros and cons. This means that the person making the decision expresses his/her real, free volition, and does not uncritically accept someone else's position that the treatment should be refused (or accepted) – the decision is the expression of his/her volition pertaining to the medical treatment. This also means that the person has, in the processes preceding decision-making, 'overcome' some, predominantly unpleasant emotions [24], which may accompany such conditions, and that his/her ratio and volition prevailed over emotional aspects of decision-making.

This is the minimum of prior knowledge that should guide a doctor when assessing the mental capacity of patient refusing medical treatment. In addition, the assessment should not neglect the patient's education, age, social and cultural background, the rate of progress of the disease that requires medical treatment, etc. Furthermore, the patient's family should be involved in the assessment, although research suggests that this rarely happens [22]. Their involvement in the assessment of the patient's moral values and motives for the refusal of treatment is invaluable, and they may even prove to be more competent for this assessment than doctor is.

It should be emphasized that the mental capacity can be affected by various mental disorders [25], which includes the cases when a person refuses medical treatment. In such case, it needs to be assessed how much these mental disorders influence or compromise the mental capacity. In other words, the assessment is made to determine the degree and manner in which these pathological mental states disturb the patient's capacity to independently and adequately take care of him/herself, his/her rights and interests [26]. Naturally, this is also taken into consideration when assessing the mental capacity of patients refusing treatment, and in practice it comprises a whole spectrum of psychiatric entities. The most frequent ones include the acute and temporary mental disorders, permanent psyciatric illness, mental retardation and mental disorders caused by an acute physical or neurological illness.

## The methodology of mental capacity assessment when medical treatment is refused

*When is assessment conducted?*

This capacity is assessed only if there is a serious doubt accompanied by indications that a patient's mental capacity is limited. These indications comprise unexpected changes of mental state, such as disorientation, problems with attention, concentration and memory. Some patient's behavior can also indicate certain mental disorder – if the patient, for example, behaves as if the decision about medical treatment does not concern him/her at all. Interpretation of the above mentioned provision of APR (art. 17, section 1) requires that the doctor must conduct the assessment whenever he/she has doubts about the presupposed capacity of an adult with contractual capacity who refuses medical treatment which saves or sustains his/her life. Only if a doctor determines that the refusal of treatment is the result of a rational decision – that there is harmony between his/her autonomy and his/her best interest – it can be concluded that the patient possesses mental capacity [27]. Once established mental capacity to refuse medical treatment must be reexamined in later treatment stages [28]. It is not a permanent state, as an individual can be in an excellent mental condition one day, and show reduced capacity the next day [29].

Refusal of treatment is in itself a great ethical challenge, especially if a person's life is at stake and his/her mental capacity is limited, or absent [30]. There is a double contradiction: it challenges the patient's right to treatment and the doctor's obligation to provide treatment while respecting the patient's autonomy. In general, one could tentatively claim that a refusal of treatment is irrational. This argument is even stronger when the doctor's values differ from the patient's. When medical treatment is refused, the treating doctor must raise the question of the patient's capacity to consent to the treatment. However, the opposing views between the doctor and the patient are not in themselves a sufficient reason to deprive the patient of mental capacity [31] nor is the acceptance of treatment the proof of mental capacity [32]. There are certain criteria that need to be respected in both situations of refusal and acceptance of medical treatment. The expression of willingness must be free and clear, communicated by a person with mental capacity. To obtain a definite answer, a doctor must provide all the necessary information regarding the diagnosis, alternative treatments and the treatments' potential side effects. Sometimes it is difficult to determine whether refusal of treatment is a result of patient's values, or of his diminished mental capacity. In any case, the fact that the doctor finds the patient's refusal of treatment unreasonable does not necessarily imply that the patient lacks mental capacity [10].

*Who conducts the assessment?*

A doctor (medical institution) - patient relationship is a type of contractual relationship [34] where each party has certain rights and responsibilities. These include the patient's decision-making, which is his/her right and responsibility – responsibility to him/herself. As decision-making pertains to a single suggested action, under a single agreement, it is logical that the doctor should conduct the assessment if there is doubt about the patient's mental capacity.

When medical treatment is refused, the law (APR) stipulates that 'the treating doctor is obliged to inform the patient about the consequences of his/her refusal of medical treatment.' The APR does not identify who should conduct the assessment of mental capacity, but practitioners are of the opinion that it should be done by the treating doctor. There is the issue of competence of practicing doctor given that there is no systematic training of doctors for the assessment of mental capacity. In addition, the broad legal definition makes the assessment even more difficult, as the criteria are mainly subjective [34]. On the other hand, one may raise the question

whether it is necessary to consult a psychiatrist to assess mental capacity in every case of refusal of treatment.

The position that the treating doctor should assess the mental capacity when treatment is refused, does not imply that the assessment cannot be done by consulting a larger number of medical professionals, most often working for the institution in which the patient is treated. In special cases, it is necessary to seek opinions of other medical specialists, outside the institution where the patient is treated (or from other wards in the same institution), especially neuropsychiatrists and medical psychologists. This type of assessment is the termed consultative assessment.

As legislation does not prescribe the form of the assessment; it can be conducted *ad hoc*, which is unfortunately the most frequent type of assessment in Serbia. Consequently, it is necessary to standardize the procedures, incorporate them into medical standards and health-care legislation either as general regulation or as regulation of an individual medical institution. Solutions should primarily be proposed by medical experts' associations, especially since no official procedures have been established. This would reduce improvisations in methodology, variations in assessment, as well as potential mistakes and damage.

### *Principles of clinical examination when assessing mental capacity in case of refusal of treatment*

It is of an utmost importance that the doctors conducting the additional examination to assess mental capacity be guided by the fundamental rules of good practice. Most significant of these is the interview about the recommended treatment conducted with the patient. It implies asking questions to clarify whether the patient understands what he/she is refusing and whether he/she can provide an explanation for the refusal. The doctor would next ask questions to assess the mental state (and disorders) and functions such as thinking and rational reasoning to make sure that the patient understands recommendations and to identify reasons (motives) for the consent to, or refusal of treatment. Naturally, all this should be put into the context of the patient's overall health status, as sudden physiological or neurological disorders can alter the patient's psychological functioning, which may have been intact prior to illness, treatment or even during a part of the treatment. As medical practice abounds in such situations, it should be taken into consideration in case of refusal of treatment. What we lack, however, in this part of the world are relevant studies which might indicate the frequency of these situations and the problems related to them, so that the solutions to potential clinical problems could be found.

The foregoing suggests that the basic assessment of mental capacity in the patient's refusal of medical treatment should comprise: valid medical assessment of the patient's overall health status, in accordance with the rules of medical profession and in relation to adequate mental functioning; focused interview with the patient about the suggested medical treatment to establish whether the patient understands the nature of treatment, its purpose and risks in case of refusal, its potential complications and side effects, as well as to make sure that he/she is capable of making an adequate decision, as an act of volition.

If these are conducted in a conscientious, medically adequate manner, majority of assessments will be done *lege artis*, efficiently and competently.

In situations when the treating doctor does not clearly understand the circumstances of assessment, certain structured methods can be applied, including the following: the Guidelines (directives) of the Association Suisse des Sciences Médicales (ASSM) which recommend the assessment of following capacities: the capacity to understand information about the suggested treatment; capacity to correctly evaluate the situation and the consequences of alternative solutions by comparing their risks and benefits; capacity to rationally evaluate obtained information applying coherent set of values; capacity to communicate his/her choice. It should be emphasized that these criteria are useful and systematic and they are also applied in procedures described above. Therefore, they are part of the subjective assessment of the examiner and not a structured questionnaire. Using the verified and standardized tests and questionnaires which enable rough assessment of mental state (being more objective than those resulting from the interview). It is our opinion that these tests can be applied by any doctor. The question is whether he/she would do it every time, which depends on the patient's mental state as observed during clinical examination.

In Serbia, most frequently used is the Mini-Mental State Examination (MMSE) [35], which can be applied by medical professionals other than psychiatrists, to determine whether there is a mental change in the patient who refuses medical treatment. If the score on this scale is such to indicate a significant cognitive damage, it is our opinion that this is an indicator requiring a consultation with a psychiatrist, except in cases where the urgency of the intervention and its life-saving nature make it technically impossible.

Needless to say, the MMSE is not the only diagnostic instrument to recognize psychologically altered states, especially when it comes to cognitive and volitional aspect of the patient's personality. The Montreal Cognitive Assessment (MoCA) [36], the Sheffield questionnaire [37] and other tests are also widely used. Although these tests are useful, it is often emphasized that nothing can replace the individual assessment conducted by a doctor [10].

### *Who should conduct an assessment and in which cases?*

There are variety of factors influencing how to conduct the assessment. Primarily, the question is whether the patient's awareness of potential consequences of a decision to consent to or refuse the treatment must play part in capacity assessment. In other words, does the mental capacity level required in the given case vary depending on the potential consequences of a decision, as suggested in a ruling of an English court: "What matters is that the doctors should consider whether at that time he had a capacity which was commensurate with the gravity of the decision. The more serious the decision, the greater the capacity required" (Re T [1992] 4 All E.R. 649) [28].

More precisely, a person can be capable of making decision about taking analgesics, yet incapable of deciding about a complicated and risky surgery [16]. Consequently, different capacity will be required when a high-risk medical treatment is to be administered, or when life-saving or life-sustaining treatment is refused. In the former case, the patient must possess the highest level of mental capacity and be able to explain his/her choice applying his/her own set of values [38]. In the latter case, it is sufficient for the patient to be able to understand the presented information in order to be considered mentally capable. To accept proportionality between the gravity of the recommended medical treatment and the degree of mental capacity seems easily defensible, but there are certain apprehensions. Some authors suggest that there is a point where proportionality stops while in some extreme situations a patient's wishes can be met regardless of his/her mental capacity [38].

Considering the foregoing discussion, in order to help those performing assessment decide whether they are entitled to do it, we tentatively propose a procedure which, in our opinion, should be incorporated into the existing regulations [40]. It is as follows: If a medical treatment implies administering procedures that do not threaten physical integrity (e.g., medication-taking, noninvasive diagnostics, noninvasive physical therapy, etc.) and/or can have mild side-effects, it is sufficient that the treating doctor assess the patient's mental capacity and make a record of it. Even when there are the observable psychiatric symptoms, whose quality and/or prominence is such that they do not violate this capacity, it is not necessary to consult a psychiatrist, although other medical professionals from the same health-care institution may be consulted (but do not have to) and involved into the assessment. Sometimes the medical treatment violates the patient's physical integrity, but does not present a serious risk to patient's health. In that case, if the patient's life is not at risk, or if he/she is not classified as a 'serious' patient, the assessment needs to be necessarily performed by an in-house consulting expert body if the suggested treatment is refused. If the expert body identifies psychiatric symptoms, or expresses doubt about the patient's psychological state, the consultative assessment of a psychiatrist is required. When recommended diagnostics and medical treatment seriously violate a patient's physical integrity, or when medical treatment is to be given to a patient whose life is at risk and who is classified as a 'very serious' patient, and the patient refuses treatment, we propose that the psychiatric assessment evaluate the patient's mental capacity regarding the treatment. This is to eliminate potential situations where the treating doctor has doubts about the patient's mental status and capacity, and thus loses precious time, since such a standardized procedure would present practical assistance to the treating doctor.

Undoubtedly, there would be situations where some necessary, especially technical requirements could not be met, but this is expected to happen less frequently. If our recommendations are inapplicable, there is always the clinical method of assessment: an adequate, conscientious, comprehensive examination. Only *lege artis* medical procedure [41] can create the preconditions for the objective assessment of mental capacity.

## Conclusion

If the patient understands presented information about the recommended medical treatment, correctly evaluates consequences of his/her choice weighing risks and benefits, he/she can be deemed to have the mental capacity to decide whether to consent to treatment. The weight of potential consequences of the suggested treatment determines the necessary capacity level in each individual case of assessment. In some cases, assessment can be conducted by the treating doctor, while in case where medical treatment with serious potential consequences is to be administered, especially if the patient refuses life-saving treatment, the assessment should be conducted by medical teams, sometimes consisting exclusively of psychiatrists. The assessment implies structured clinical approach, based on a doctor's personal assessment as well as on the standardized procedure. To avoid potential consequences both for the patients and the doctors in case of inaccurate assessment of this capacity, certain standardization of assessment procedure should be adopted.

## Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, through Contract No. 179046.

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Received on October 26, 2017.
Revised on November 24, 2017.
Accepted on December 13, 2017.
Online First December, 2017.

GENERAL REVIEW

UDC: 616.12
https://doi.org/10.2298/VSP170701161S

# Arterial stiffness as predictive factor of cardiovascular diseases

## Arterijska krutost kao prediktivni faktor kardiovaskularnih oboljenja

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**Key words:**
**antihypertensive agents; arteries; cardiovascular diseases; elasticity; equipment and supplies; hypertension; pulse wave analysis; risk assessment.**

**Ključne reči:**
**antihipertenzivi; arterije; kardiovaskularne bolesti; elastičnost; oprema i pribor; hipertenzija; pulsni talas, analiza; rizik, procena.**

### Introduction

Understanding the basic hemodynamic principles is necessary for the assessment of arterial stiffness and its possible clinical practice. Hydraulic and elasticity theory was established by Young in 1840, Moens in 1878, and Korteweg in 1878 [1]. The main determinants of pulse wave velocity (PWV) relates to the velocity of pulse wave travel in a vessel to the distenbility of that vessel [2, 3]: (PWV) = $\sqrt{(Eh/2R\rho)}$, (E is the Young's modulus in the circumferential direction, h is the wall thickness, R is the vessel radius and $\rho$ is the density of fluid).

Later, the doctors-physiologists, Marey in 1860, Mahomed in 1872 and Mackenzia in 1902, developed various types of Sphygmocor and, thus, made a significant progress in pressure waveform analysis [4]. Clinical application was discovered by Safar and O'Rourke [5, 6], which turned out to be very useful in the prognosis of the outcome and the correction of therapy.

In healthy persons, the peripheral arteries are stiffer than the central and that leads to an increase of amplitude of pulse wave in blood vessels, from heart to periphery, which is known as pressure amplification [6, 7]. The stiffness of mid-size arteries is assessed by a vasomotor tone which depends on the endothelial function or the sympathetic nervous system [8–10] or on renin-angiotensin system [11].

Elastic properties of arteries vary along the arterial tree, with more elastic proximal and stiffer distal arteries. In humans, PWV increases from 4–5 m/s in the ascending aorta to 5–6 m/s in the abdominal aorta, and 8–9 m/s in the iliac and femoral arteries [11]. This difference in the arterial stiffness has the significant physiological and pathophysiological consequences.

Due to an increase of pulse pressure (PP) between the central and peripheral arteries, brachial PP should not be used as a replacement for aortic or carotid PP, especially in younger persons.

The stiffness of the common carotid artery is approximately six times higher in 70-year old persons than in a 20-year old person [6, 12]. In the older patients with hypertension or diabetes, the carotid arteries may become stiffer than the femoral or radial arteries which become slightly stiffer with age, or due to hypertension.

### Noninvasive assessment of arterial stiffness

The regional and local arterial stiffness may be measured directly in a noninvasive manner, in various locations along the arterial tree and it is based on measuring the parameters which are directly connected to the arterial stiffness.

#### *Local assessment of arterial stiffness*

The arterial stiffness can be assessed locally through the use of ultrasound device which also provides precise measures of intima-media thickness (IMT) which allows the assessment of elastic properties of arterial vessel according to the Young's elastic modulus [13]. The assessment of carotid stiffness and thickness is important for the development of atherosclerosis. The local arterial stiffness of deep arteries such as aorta may also be measured by the magnetic resonance imaging (MRI), but this is not commonly applied in routine practice. Aortic PWV assessed by transformation of the brachial pressure waveform did not show a significant difference comparing with the cardiac magnetic resonance – derived transit time method [14].

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Although the carotid-femoral PWV and carotid stiffness provide similar data on how age affects the stiffness of big arteries in healthy persons, this is not the case with the persons with hypertension and/or diabetes. With age and other cardiovascular risk factors, the aorta gets stiffer than the carotid arteries and that is why the aortic and carotid stiffness can not be used as the variable predictors of the high-risk patients [15].

*Regional assessment of arterial stiffness*

Aorta is the main blood vessel of interest in the assessment of regional arterial stiffness, but all of the arterial branches have their own impact. *Arteria brachialis*, where the pressure is most often measured, and the lower extremity arteries get especially altered by atherosclerosis. Measurement of a local carotid stiffness may also provide a significant prognostic information since the carotid artery is often the place where atheroma appears.

*Pulse wave velocity measuring*

The PWV measurement is the simplest, noninvasive method for the assessment of arterial stiffness. Carotid-femoral PWV is a direct measurement and is considered a golden standard for the assessment of arterial stiffness, but it is important to measure precisely the distance between the carotid and femoral artery, because even small mistakes may affect the absolute value of PWV [16–18]. Measuring along the aorta or aortoiliac path is clinically the most relevant, since the aorta and its first branches are connected to the left ventricle and therefore are the most responsible for the majority of the arterial stiffness effects. Contrary to that, the PWV measured outside of aortic system, that is, in the upper extremities (brachial PWV) or lower extremities (femorotibial PWV) do not have a good predictive value in the patients with terminal kidney disease [19].

It is difficult to register the femoral pressure waveform in persons with metabolic syndrome, excess body mass, diabetes and peripheral arterial disease [20]. In case of aortic, iliac, or stenosis of proximal segment of femoral artery, the pressure waveform may be delayed or reduced. Abdominal obesity, especially in men, and large breasts in women may cause an inaccurate measuring of distance [21].

**Arterial stiffness measuring devices**

SphygmoCor® is a tonometric device; PWV is calculated on the basis of successive waves produced in short time interval in two arterial locations (most often the carotid and femoral artery) through use of R waves in the electrocardiogram (ECG) for the calculation of the delay [22].

Measurements are most often done at the root of the left subclavian artery (suprasternal space on the skin) or close to abdominal aortic bifurcation (at the level of umbilicus). Transit time is automatically calculated by the recognition of the beginning of pulse. This method is used for the assessment of predictive value of aortic PWV for cardiovascular events in the patients with diabetes and it gives more precise assessment of aortic PWV when compared to the carotid-femoral PWV. The aortic brachial PWV is an important predictor of cardiovascular events in the patients with hypertension [23].

TensioMed Arteriograph® is a device for measuring stiffness of arterial blood vessels that works according to the patented oscillometric principle. Data received via arteriograph [augmentation index (AIx), PWV, central systolic blood pressure (SBP) and PP] match the data received via the brachial artery catheter. Comparison was also made with Applanation Tonometer Sphygmocor® and no significant difference was observed. For the assessment of the arterial stiffness parameters with these devices, it is necessary to prepare the patient adequately: the use of alcohol 10 h before and the use coffee 3 h before the measurement are strictly forbidden; patients must be in a semisupine position 10 min before the measurement. The assessment is performed in supine position at the room temperature of 22 ± 1°C. The arteriograph device measures aortic PWV (PWVao), AIx and central blood pressure (SBPao) values simultaneously with the peripheral blood pressure. By inflating the cuff on the upper-arm to suprasystolic pressure the brachial artery becomes occluded. The brachial flow is stopped, therefore the brachial wall characteristics are excluded (no significant wall movement), consequently the gained information relate to the systematic circulation. For calculating the arterial function parameters the recorded pulse waveform is analyzed and the characteristic points of the first and reflected waves are determined. The true aortic length is estimated with the jugulum symphysis distance (Jug-Sy).

The optimal values are: AIx < -30%, PWV < 7 m/s. The pathological values are: AIx >10%, PWV > 12 m/s.

Besides the classic 24-h blood pressure measurement, the latest device with HMS CS program combined with the Mobil-O-Graph® also has an integrated system for 24-h ambulatory monitoring of arterial stiffness through the use of oscillometric method. The Mobil-O-Graph® measures standard blood pressure parameters, central aortic pressure, central PP, AIx standardized to a heart rate of 75 beats through empirical regression (AIx@75), PWV, cardiac output, cardiac index, and total vascular resistance reflection magnitude. Biolectrical impedance gives us these values at present time [24, 25]. 24-h monitoring both of arterial stiffness and hemodynamic enables the provision of more precise parameters in cardiovascular prediction. There is no specific preparation of patients for wearing this device and one advantage is that all parameters are monitored 24 h during usual the daytime and nighttime values. In addition, the therapeutic effect of medicine can be assessed much better, with a much better prognostic effect on damages of target organs.

The Mobil-O-Graph® parameters are shown in Figure 1.

There is no difference in the value of central aortic pressure measured by using the oscillometric noninvasive method with the Mobil-O-Graph® device and the tonometric method with the SphygmoCor® device. The Mobil-O-Graph® combines the advantages of assessing brachial pressure and central blood pressure in one measurement [20]. As with other populations, the acceptability of Mobil-O-Graph® and SphygmoCor® is evident for central SBP and AIx@75 in the

dialysis patients; PWV is slightly underestimated by the Mobil-O-Graph [22]. Noninvasive 24-hour assessment of blood pressure level and blood vessel stiffness is of prognostic value for a cardiovascular risk [26]. According to the SAFER study, the left ventricular hypertrophy (LVH) is more associated with 24-hour aortic pressure than with 24-hour brachial pressure in the patients with hypertension [27]. Aortic pressure correlates better with all, especially cardiovascular mortality caused by the brachial artery pressure [28].

## Noninvasive assessment of arterial stiffness parameters

### *Central pulse wave analysis*

Arterial pressure waveform consists of the pulse wave initiated by the ventricular contraction and reflected wave. The waves are reflected from periphery, mostly from the branching point. In elastic blood vessels, due to the low PWV, reflected wave has a tendency to return to the aortic root during diastole. In case of stiff arteries, PWV increases and reflected wave arrives to the central artery earlier, where it acts as an enhancement to the initiated wave and increases systolic pressure. This phenomenon may be quantified through AIx which is defined as a difference between the second and first systolic peak (P2-P1) expressed as a percentage of PP (Figure 1) [4, 16]. Regardless of high PWV, changes in the location of reflection may affect AIx. In clinical research, the main AIx determinants are not only diastolic blood pressure and height, but also age and aortic PWV.

Central AIx and central PP showed an independent predictive value when it comes to all-cause mortality in the dialysis patients [28] and cardiovascular events in the patients who underwent percentaneous coronary intervention, as well as in the patients with hypertension in the CAFE study [29].

Figure 2 shows AIx which is defined as difference between the second and first systolic peak (P2-P1) expressed as a percentage of PP.

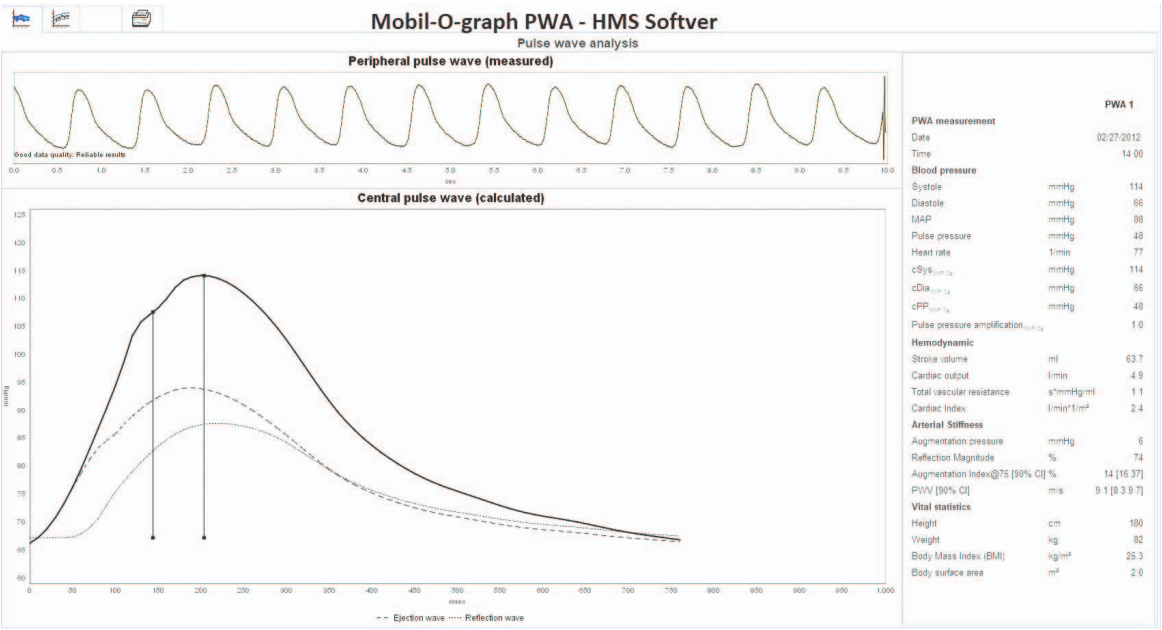


**Fig. 1 – Mobil-O-Graph parameters**
**PWA – pulse wave analysis;MAP – mean arterial pressure; cSys – central systolic pressure; cDia – central diastolic pressure; cPP – central pulse pessure; PWV – pulse wave velocity.**

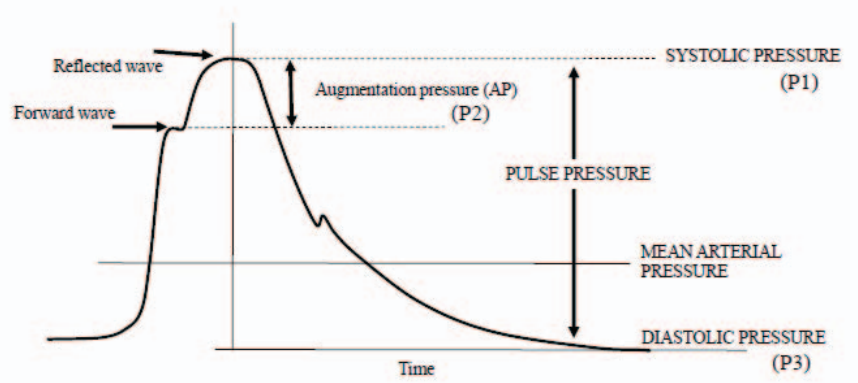


**Fig. 2 – Augmentation Index (AIx) – which is defined as difference between the second and first systolic peak (P2-P1) expressed as a percentage of the pulse pressure.**

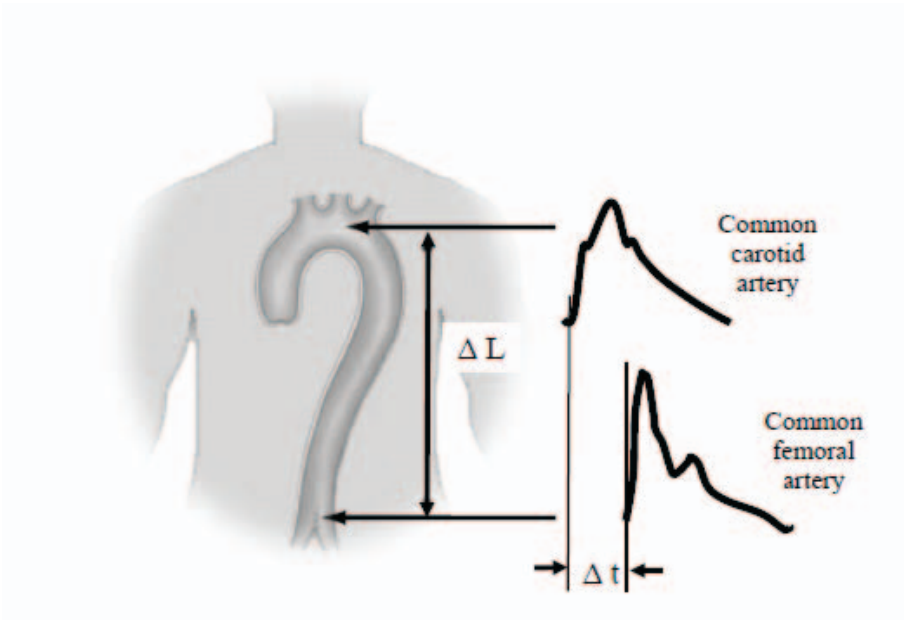


**Fig. 3 – The foot to foot method for measurement of carotid-femoral pulse wave velocity.**

*Central and peripheral systolic and pulse pressure*

Peripheral pressure SBP and PP (measured on the brachial artery) should not be equated with central SBP and PP measured on carotids, since peripheral pressure is higher than the central and PP, especially in younger persons, due to the less stiff central artery in younger persons.

*Central pulse pressure, augmentation index and arterial stiffness*

Central SBP and PP, AIx and PWV increase with age, hypertension, diabetes mellitus and hypercholesterolemia, and they are connected with damage to target organs (LVH, microalbuminuria, carotid plaques, and endothelial dysfunction). Central SBP, PP and AIx depend on the speed at which the wave travels, amplitude of the reflected wave, point of reflection, duration and size of ventricular ejection, especially in relation to heart rate and ventricular contractility [30], while aortic PWV which is the wave propagation speed, presents the internal arterial stiffness according to the Bramwell-Hill equation (Figure 2). Pathophysiological conditions and medicines can change central PP and AIx without changing aortic PWV, which shows the dominant influence of the reflecting wave, heart rate or ventricular ejection, without the change of arterial stiffness [30–31]. AIx is much more sensitive to heart rate than PWV. In the Anglo-Cardiff Collaborative Trial conducted in the general population, it is shown that younger than age of 50 years affects AIx more than it affects PWV in the people below 50 years of age, but it is the opposite after the age of 50 years [34].

Central pressure and AIx have a significant predictive value in the patients with hypertension, coronary disease and kidney diseases [35].

Figure 3 shows the foot to foot method for measurement of carotid-femoral PWV.

**Clinical application**

*Pathophysiology*

The stiffness of vascular wall depends on two main fiber proteins which are part of its composition: collagen and elastin. Excessive production of abnormal collagen and reduction of normal elastin plus their inadequate spatial organization lead to arterial stiffness [36].

There are many risk factors for the increased arterial stiffness: age, low birth weight, menstrual cycle, menopause, lack of physical activity, genetic predisposition to hypertension, diabetes, myocardial infarction, genetic polymorphism, obesity, smoking, hypercholesterolemia [37], glucose intolerance, metabolic syndrome, hyperhomocysteinemia, high levels of C-reactive protein (CRP). Also, cardiovascular diseases, such as coronary disease, congestive heart failure [2], stroke, as well as non-cardiovascular diseases, such as moderate chronic kidney disease stage 3 [38], rheumatoid arthritis, systemic vasculitis and systemic lupus erythematosus [30] represent risk factors for the increased arterial stiffness**.**

The arterial stiffness and reflection of waves are important for an increase of systolic pressure in elderly persons, and they play an important role in the occurrence of cerebrovascular stroke and myocardial infarction.

The arterial stiffness causes premature return of reflected wave in early systole, which increases central PP and then systolic blood pressure which increases left ventricular burden and increases the need for oxygen. In addition, the arterial stiffness is combined with LVF [27], which is a significant risk factor for coronary disease in the normotensive and hypertensive patients. The increase of central blood pressure and decrease of diastolic BP can directly cause subendocardial ischemia. The increase of aortic stiffness, together with aging and risk factors for cardiovascular diseases, is caused by various mechanisms including the degradation of elastic

fibers, collagen accumulation, fibrosis, inflammation, medial smooth muscle cell necrosis, calcification and diffusion of macromolecules across the arterial wall [29]. The increased arterial stiffness may increase the risk of stroke through several mechanisms, including the increase of central PP, influence on remodeling of intra- and extracranial arteries, increase of carotid wall thickness, and development of stenosis and plaques [29, 30, 39], with plaque rupture and damage to the white matter of brain. In addition, the coronary disease and heart weakness with high PP and the arterial stiffness are risk factors for the occurrence of stroke.

*Routine application of arterial stiffness*

The aortic stiffness has an independent and more significant predictive value than classical risk factors for all-cause and cardiovascular mortality in the patients with hypertension, type 2 diabetes, patients on dialysis and elderly persons, since it shows damage to blood vessel through risk factors during the longer time period [22].

Aortic PWV has a better predictive value than the classical cardiovascular risk factors. Central AIx and PP showed an independent predictive value for all-cause mortality in the patients on dialysis, or after kidney transplantation and for cardiovascular events in the persons with hypertension and coronary disease, after percutaneous coronary intervention [40]. It was shown in meta-analysis that the aortic stiffness expressed as aortic PWV is a strong predictor of future CV events and all-cause mortality. The predictive ability of arterial stiffness is higher in the subjects with a higher baseline cardiovascular risk [41, 42]. Also, aortic PWV may enable better identification of the high-risk populations that might benefit from more aggressive cardiovascular disease risk factor management [43].

*Predictive value of pulse wave velocity for cardiovascular event reduction*

An important question is whether the reduction of PWV is associated with the accompanying reduction of cardiovascular events, regardless of the normalization of classical risk factors?

The reduction of arterial stiffness may indicate an actual reduction of damage to blood vessel wall, while blood pressure, glycaemia and lipids may be normalized in several weeks through medical therapy, resulting in a significant reduction of cardiovascular risk score, but without the improvement of atherosclerotic lesion and the arterial stiffness which require a long-term correction of biochemical parameters. Therefore, a temporary dissociation between the reduction of cardiovascular risk factor and further present arterial stiffness is to be expected.

It is still to be proven whether reduction of central PP is associated with the accompanying reduction of cardiovascular events, regardless of normalization of classical risk factors. There is indirect evidence of this. In the REASON study [38, 44], only the combination of perindopril/indapamide managed to significantly reduce reflection of carotid wave with the resulting relative reduction of central SBP and PP and subsequent reduction of LVH [42] as opposed to no reduction of carotid PP and LVH in the patients who used atenolol in their therapy. The CAFE study [40] and ASCOT study [45] showed that central pressure, AIx and PP were the independent predictors of cardiovascular events in the hypertensive patients and that reduction of central SBP and PP was higher in the amlodipine/perindopril group than in the atenolol/thiazide group, despite of similar reduction of SBP and PP of brachial artery.

**How to reduce arterial stiffness (arterial stiffness therapy)**

Nonpharmacological and pharmacological therapies have an important place in the arterial stiffness reduction.

Nonpharmacological therapy includes a regular moderate physical activity, weight loss, reduced salt intake, moderate consumption of alcohol, garlic powder, alpha linoleic acid and fish oil, as well as hormone replacement therapy [44–48].

Pharmacological therapy includes the application of antihypertensive medicines and medicines for the cardiac insufficiency treatment: diuretics, beta blockers, angiotensin-converting-enzyme (ACE) inhibitors, type 1 angiotensin-receptor blockers (ARBs) and calcium antagonists, nitrates, hypolipidemic medicines such as statins and fibrates, antidiabetics such as thiazolidinediones as well as sildenafil [17, 49].

The majority of antihypertensive drugs have the main influence on the dynamic component of arterial stiffness and in some part on the structural component in the arterial wall remodeling [18].

In general, the renin-angiotensin system inhibitors are superior to all other antihypertensive drugs in reducing arterial stiffness. One reason is the profibrotic action of the renin-angiotensin system, as the turnover of the extracellular matrix in the arterial wall per se leads to a change in the properties of the vessel [50, 51].

The REASON study showed the positive effects of ACE inhibitors on the arterial stiffness, especially on AIx. The effects lasted even after nine months of treatment [44, 52]. Positive effects were shown for most drugs in this group, including for lisinopril [53].

If reduction of AIx is in focus, losartan in the LIFE [54], OPTIMAAL [55] study and candesartan [56, 57] showed positive effect on reduction. Some others ARBs valsartan (VALUE study) [58, 59] and telmisartan [60] reduce AIx and PWV, but increase PP.

Figure 4 shows the case from our practice (a – before therapy with losartan and b – six months on losartan therapy.

Beta-blockers without the vasodilating effects have a weaker effect on the arterial stiffness and central pulsatile hemodynamics than vasodilating drugs of other antihypertensive groups. The mechanism of action is through the heart rate reduction, as this influences the viscoelastic properties of the arterial wall. Reduced heart rate also leads to increased wave reflections, a lower reduction in aortic than brachial systolic blood pressure and reduced PP amplification. Pe-

ripheral vasoconstriction, achieved by atenolol, is an additional mechanism responsible for the negative effect on wave reflections [61, 62]. New agents such as nebivolol, which have vasodilating effects, seem to be more effective in improving central pulsatility. These effects appear to be related to their ability to donate nitrit oxide (NO), which dilates the small resistance arteries. The effects observed lead to PP amplification, but AIx reduction [61–63].

Calcium channel blockers also lower PWV and reduce wave reflections, but to a lesser degree than renin-angiotensin inhibitors. The largest amount of evidence is for amlodipine. In the CAFE study 64, it was showed to reduce central blood pressure more than peripheral blood pressure; it amplified pulse pressure and reduced AIx.

Diuretics seem to have no beneficial effect on pulsatile hemodynamics. Hydrochlorothiazide showed a neutral effect on reduction of central blood pressure and a neutral effect on PP amplification [65, 66].

### Arterial stiffness and damage to target organs

The arterial stiffness also provides data on the damage of target organs, which is of a great importance for the assessment of total cardiovascular risk in the patients with hypertension.

In case of primary coronary event in the hypertensive patients, the value of aortic PWV is much more important for the low-risk patients (first or second tertile of the Framingham risk score) than for the high-risk patients (third or fourth tertile), which shows that the population with low to medium risk benefits the most from the value of PWV [30].


**Fig. 4 – The case: a) before therapy with losartan; b) six months on losartan therapy, consecutively.**

**Sys – systolic; Dia – diastolic; PP – pulse pressure; MAP – mean arterial pressure; SBPao – control systolic blood pressure; HR – heart rate; AIx – augmentation index; PPao – central aortic pulse pressure; ABI – ankle brachial index; ED – ejection duration; SD – standard deviation of the beat to beat measured aortic pulse wave velocity values; PWVao – aortic pulse wave velocity; RT – return time.**

The latest results showed that the increased aortic stiffness is an independent predictor of major adverse cardiac and cerebrovascular events (MACCE) after acute ST-elevation myocardial infarction. The assessment of aortic stiffness in addition to classical risk factors significantly improved an early risk stratification [67].

Concerning diastolic dysfunction, the arterial stiffness was correlated with the elevated left ventricular filling pressure and it was shown that the increased level was associated with an elevated left ventricular filling pressure in the patients with the preserved systolic function. It was hypothesized that the increased arterial stiffness is of pathophysiological relevance for the diastolic dysfunction [68].

**Conclusion**

The existing European and American [70] recommendations for the diagnosis and treatment of hypertension define LVH and albuminuria as evidence of target organ damage, as well as the arterial stiffness and reflection of waves.

The assessment of arterial stiffness and central pressure should be considered as a recommended test for assessment of cardiovascular risk, especially in the patients with the damages on the target organs that went undetected during the routine examinations. According to the recommendations of the American Society of Hypertension (ASH), the assessment of blood vessels stiffness should be performed by a hypertension specialists in the hypertension centers. Introduction of this diagnostic procedure in routine practice is still under the consideration [70].

According to the European Society of Hypertension (ESH) guidelines of 2013, the stiffness of large arteries and wave reflection phenomenon are the important pathophysiological indicators of isolated systolic hypertension, and, together with PP, they increase with age [28, 71]. Carotid-femoral PWV is a golden standard for measuring the aortic stiffness [71]. According to the common guidelines issued by the European Society of Hypertension and European Society of Cardiology (ESC) [72], the PWV value of > 12 m/s indicates a significant deterioration of aortic function in the middle-aged hypertension patients. According to the recent research, due to the use of correct carotid-femoral distance and taking into consideration 20% anatomically shorter pulse wave distance, the PWV value of > 10 m/s indicates the increase of aortic stiffness [73]. Central pressure, especially central SBP and PP are more important predictors of occurrence of cardiovascular events and assessment of cardiovascular risk from brachial pressure [72]. For the valid measuring of central pressure, you can use not only the tonometric devices (SphygmoCorCP®) but also the oscillometric ones (SphygmoCor XCEL®) [74], as well as the 24-hour arterial stiffness monitoring device, the Mobil-O-Graph® [18].

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Received on July 01, 2017.
Revised on October 10, 2017.
Accepted on October 30, 2017.
Online First November, 2017.

UDC: 616.379-008.64-06:616.61
https://doi.org/10.2298/VSP190820079I

# Renin-angiotensin and kallikrein-kinin systems in diabetic renal damage

## Renin-angiotenzin sistem i kalikrein-kinin sistem u dijabetesnom bubrežnom oštećenju

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**Key words:**
**diabetic nephropathies; hypertension; angiotensin-converting enzyme inhibitors; angiotensin receptors antagonists; treatment outcome.**

**Ključne reči:**
**dijabetesne nefropatije; hipertenzija; angiotenzin-konvertujući enzim, inhibitori; angiotenzin receptori, antagonisti; lečenje, ishod.**

### Introduction

Erdös [1] recognized that biologically active peptides generated by the renin-angiotensin and kallikrein-kinin systems, e.g., angiotensin II, bradykinin, and kallidin (Lys-bradykinin), are so rapidly metabolized and inactivated that their transient effects would never be useful medications, but the role of these peptides in certain physiological or pathological conditions could be determined by agents that directly block their effects or inhibit either their enzymatic degradation or production. This concept enabled investigators to discover captopril and later other angiotensin I converting enzyme (ACE) inhibitors and turn them into clinically useful drugs for treatment of hypertension and related cardiovascular diseases. The aim of this presentation is to assess a role of the renin angiotensin system (RAS) and the kallikrein kinin system (KKS) in a diabetic renal damage.

### Renin-angiotensin and kallikrein-kinin systems

With the finding that ACE is identical to kininase II, an enzyme that inactivates bradykinin by removing the C-terminal $Phe_8$–$Arg_9$ dipeptide, it has become clear that ACE is involved both in the RAS and KKS [2–4]. The dual action of ACE converts both the inactive decapeptide Ang I to the hypertensive octapeptide Ang II, and inactivates potent hypotensive kinins, bradykinin and kallidin, into inactive metabolites (Figure 1).

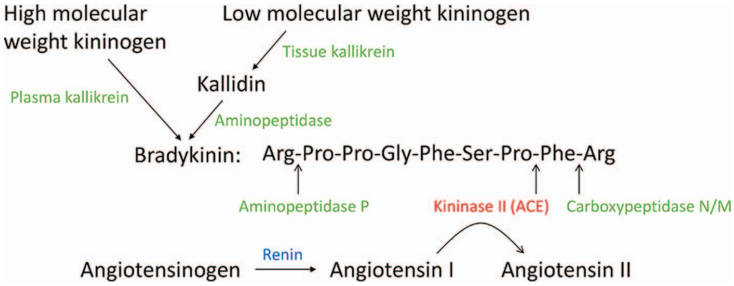


**Fig. 1 – Peptides and peptidases of the kallikrein kinin system (KKS) and a portion of the renin angiotensin system (RAS). The enzymes in the KKS are shown in green, while renin is shown in blue; angiotensin converting enzyme (ACE), which acts in both systems, is shown in red.**
**Credit *Journal of Biological Chemistry* [4].**

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The RAS is a complex system, including about twenty peptides and peptidases, and six receptors [5, 6]. This system operates at systemic and cellular levels, and it is an important volume regulator in vertebrates. Ang II is a potent vasoconstrictor that contributes to regulation of blood pressure, fluid volume, electrolytic balance, and inflammation. Its bioavailability depends on activity of two enzymes: renin, which is produced by the renal juxtaglomerular cells and acts on angiotensinogen to form Ang I and the ACE. Once formed, the inactive Ang I is converted to the active Ang II by ACE. The released Ang II then activates two types of Ang II receptors, AT1 and AT2. The AT1 receptors induce vasoconstriction, including the renal arteries, while AT2 receptors may oppose this effect. In addition to the beneficial effects of decreased blood pressure, the AT2 receptors are involved in nitric oxide and cyclic guanosine monophosphate (cGMP) production, inhibition of apoptosis, and anti-proliferative action which is beneficial in reducing tissue damage.

ACE is unevenly distributed in the vascular tissue [7]. The lungs have the highest and the kidneys have the lowest amount compared to other organs, including the heart. Individuals with genetically high ACE levels have the higher risk for renal and cardiac damage; this is especially true for diabetic subjects, due to a greater inactivation of bradykinin than Ang II production by this enzyme [8].

All components of the KKS exist only in mammals; thus the system evolved quite late during evolution [9]. Humans have more tissue kallikrein, which mostly originates from the kidney, than the circulatory enzyme; in rodents, kallikrein originates primarily from the salivary glands.

Two kallikrein enzymes differ in the molecular weight, amino acid sequence and immunogenicity [4]. Plasma kallikrein, also known as Fletcher factor, releases the nonapeptide bradykinin (Arg1-Pro2-Pro3-Gly4-Phe5-Ser6-Pro7-Phe8-Arg9), while tissue kallikrein (K1) releases Lys-bradykinin (kallidin). Kallidin can be converted to bradykinin by aminopeptidase (Figure 1). Kallikrein produces kinins by cleaving a high molecular weight (110 kDa) kininogen substrate (HMWK, also known as Fitzgerald factor or factor XII) as well as the low molecular weight (LMWK, 50–68 kDa) kininogen substrate. Both types of kininogen substrates (high and low molecular weight) are synthesized in the liver and are present in high concentrations in plasma.

Kinins act through two types of receptors, B1 and B2. The activation of B2 receptors increase vasodilation, vascular permeability and sweating. The B2 receptors also increase intracellular calcium in the smooth muscle and endothelial cells and activate signaling cascades (e.g., the phospholipase A2). The B1 receptors are normally expressed only at low levels, but the tissue injury, inflammation, ischemia, chronic hyperglycemia, or endotoxin can induce their synthesis. They are also stimulated by ACE inhibitor therapy [10]. Kinins release nitric oxide, prostacyclin and tissue plasmin activator (t-PA) from the endothelial cells. These peptides are protectors against oxidative stress and organ damage in the heart and kidney. Their increase by the ACE inhibitors and vasopeptidase inhibitors is beneficial both in cardiovascular diseases and nephropathy. Icatibant is a specific antagonist of B2 receptors that is used to treat acute attacks of hereditary angioedema. The half-life of kinins in the circulation is short, less than 15 seconds [11]. However, because of their potent effects on various systems, they may be important for end-organ protection, particularly in the kidney. Locally generated kinins could thus help to prevent diabetic renal damage, known as diabetic nephropathy (DN).

**Diabetic nephropathy**

DN is a major cause of the end-stage renal disease. It affects approximately one-third of individuals with diabetes mellitus and is associated with the great morbidity and mortality [12]. Chronic hyperglycemia causes hypertrophy of glomeruli, thickening of renal basement membranes, microalbuminuria, glomerulosclerosis, tubular and interstitial fibrosis and the reduction in glomerular filtration [13]. It takes about ten years of this diabetic condition for development of albuminuria. Hyperglycemia and hypertension accelerate the progression to end-stage renal disease. DN may cause nephrotic syndrome, especially in the elderly patients. As of now, there is no cure for DN available, but it is possible to slow its progress. The established DN treatment includes strict glycemic control, blood pressure control and RAS blockade.

In diabetes, endothelial ACE increases and local generation of Ang II rises, but at the same time, the kinin levels decreases more rapidly. The diabetic patients with the genetically increased ACE levels have a higher risk for nephropathy and cardiovascular damage, including neuropathy and retinopathy. The initial observations suggested that the genetically higher ACE levels slightly increase the risk for development of myocardial infarction, but in the diabetic patients the risk is much greater [14]. Many studies [15–20] indicate that the genetically increased ACE production in diabetes, of both type 1 and type 2, poses a risk for nephropathy.

ACE inhibitors and AT1 receptor blockers (ARBs) are used therapeutically to block the RAS to decrease morbidity and mortality in the patients with chronic heart failure. The use of these drugs delays progression of vascular lesions, controls hypertension, diabetic nephropathy, and nondiabetic chronic renal disease [21]. Correcting the imbalance between the RAS and the KKS with ACE inhibitors restores cardiovascular homeostasis and helps to reduce the damage by various cardiovascular diseases [22]. Many clinical studies using ACE inhibitors and ARBs separately, or in the combination, explored the role of the RAS in diabetic nephropathy. The RAS is thought to promote nephropathy mainly by releasing Ang II to act systemically and at the cellular level. The effects include: constriction of arteriolar smooth muscle, increased vascular pressure, inflammation, enhanced cell growth, migration and apoptosis.

Various ACE inhibitors and ARBs are used successfully in clinical practice. In some conditions ARBs are superior to ACE. For example, ARBs are more effective in diabetic proteinuria than ACE inhibitors, as it was shown in the Diabetes Exposed to Telmisartan and Enalapril (DETAIL) study. Combined blockade of the RAS by the ACE inhibitor

and ARBs is more beneficial in reducing proteinuria, blood pressure and vascular morbidity and mortality [22]. Many studies of the RAS blockade in diabetic nephropathy have shown a significant improvement, although none completely blocked the harmful peptides. From the available information provided by these clinical studies, it is clear that ACE inhibitors and/or ARBs remain the first line among pharmacological treatments in diabetic nephropathy.

**Future therapeutic development**

Due to their dual action, ACE inhibitors obstruct both Ang II release and bradykinin inactivation, producing a beneficial effect on diabetic nephropathy independent of their effects on the blood pressure and Ang II levels. The KKS is clearly involved in the pathophysiology of this disease. In fact, an increased ACE activity has a greater effect on the kinin levels than on the Ang II levels. Many studies were done to determine which kinin receptors are involved in the renal protection. The studies with the mice lacking either the B1 or B2 receptor, or both [23], coupled with the observations in the human patients revealed the important role of B1 receptors [14]. In addition, a selective blockade of the B2 receptors resulted in the increased B1 receptor activation by kinins. Thus, it would seem that the kinin B1 receptor agonists might provide an additional armament against diabetic nephropathy and end-organ damage in other tissues. By reducing the inactivation of kinins, ACE inhibitors could further enhance the therapeutic effects.

Peptide analogs of kinins resistant to the actions of peptidases already have been synthesized [14, 24, 25] but they are still used primarily in research and applied only intravenously, or through osmotic mini-pumps. Among the future difficult endeavors, there should be a goal to discover the convenient B1 receptor agonist that could be ideally given orally.

Another approach would be to induce kallikrein production by the genetic modification of stem cells, or progenitor cells with a kallikrein gene designed to enhance their viability and proliferative, migratory and functional properties. Thus, it could be a novel therapeutic target in the treatment of a wide range of cardiovascular, cerebrovascular and renal disorders [26]. If successful, gene editing would be an important treatment for the type 1 diabetes elimination.

**Note**

I devote this article to Ervin G. Erdös (96), my teacher and good friend. We have collaborated in research for several decades [4]. He visited the former Yugoslavia several times where he presented seminars (Sarajevo, Tuzla, Sombor, and Belgrade) and stimulated local scientists to study metabolism and activity of vasoactive peptides.

**Acknowledgment**

The author is tankful to Dr. Alice R. Johnson for reading the manuscript.

**Disclosure**

The author declared no competing interest.

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Received on August 20, 2019.
Accepted on August 22, 2019.
Online First September, 2019.

CASE REPORTS


UDC: 615.099:615.2/.3]:616-08-039.76
https://doi.org/10.2298/VSP170926164S

# Extracorporeal life support for severe cardiogenic shock induced by diltiazem intoxication

## Vantelesno održavanje života kod teškog kardiogenog šoka izazvanog intoksikacijom diltiazemom

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**Abstract**

**Introduction.** Management of cardiogenic shock caused by severe drug intoxication is always challenging. In case of multidrug intoxication, a result, despite aggressive medical therapy, is often unpredictable. Utilization of extracorporeal life support devices in these cases has been suggested and reported results are promising. **Case report.** We presented a case of profound cardiogenic and distributive shock caused by suicidal intoxication with diltiazem and anionic surfactant ingestion in a 36-year-old woman. The patient ingested more than 90 tablets of diltiazem of 90 mg (ingested dose of 8.1 g), and 4 pieces of household toilet refresh agent containing anionic surfactant. During the admission, systemic blood pressure was 65/40 mmHg, heart rate 45 beats per minute, with signs of metabolic acidosis. The patient underwent several repeated gastric lavages. Emergent fluid resuscitation, calcium gluconate, insulin and vasopressive agents (dopamine and noradrenaline) infusions were administered with negligible effect. Due to progressive and refractory cardiogenic shock with signs of multiorgan failure, a decision was made to put the patient on venoarterial extracorporeal membrane oxygenator. Immediately after starting the extracorporeal membrane oxygenation, diuresis was established. During the next 36 h, an adequate end-organ perfusion was achieved with complete reversal of multiorgan failure. After the successful restoration of all major organ functions, the patient was successfully decannulated and discharged from the hospital after 10 days in a good condition. **Conclusion.** In severe cases of refractory cardiogenic and distributive shock due to diltiazem and other poison intoxication, venoarterial extracorporeal membrane oxygenation could allow additional circulatory support providing the bonus time for endogenous clearance of toxins. Venoarterial extracorporeal membrane oxygenation could be used in conjunction with the optimal medical therapy aiming to the restoration of end-organ perfusion and allowing for intrinsic drug and toxin metabolism and natural elimination.

**Key words:**
**calcium channel blockers; extracorporeal membrane oxygenation; poisoning; pulmonary edema; shock, cardiogenic; treatment outcome.**

**Apstrakt**

**Uvod.** Lečenje kardiogenog šoka izazvanog teškim trovanjem lekovima uvek predstavlja veliki izazov. U slučaju polimedikamentnog trovanja, ishod je, uprkos agresivnoj medikamentnoj terapiji, nepredvidiv. Predložena je upotreba uređaja za vantelesno održavanje života u takvim slučajevima i to sa obećavajućim rezultatima. **Prikaz bolesnika.** Prikazali smo bolesnika sa teškim kardiogenim i distributivnim šokom izazvanim suicidalnim trovanjem – ingestijom diltiazema i anjonskog surfaktanta kod 36-godišnje žene. Bolesnica je progutala više of 90 tableta diltiazema od 90 mg (ukupna doza 8,1 grama) i 4 "kuglice" sredstva za osvežavanje toaleta koje sadrži anjonski surfaktant. Tokom prijema, sistemski krvni pritisak bio je 65/40 mmHg, frekvencija srčanog rada 45/min, sa znacima metaboličke acidoze. Bolesnica je podvrgnuta ponavljanim gastričnim lavažama. Urađena je hitna nadoknada volumena, primenjen je kalcijum glukonat, kao i insulin i vazopresorni lekovi (dopamin i noradrenalin) sa zanemarljivim efektima. Usled progresivnog i refraktornog kardiogenog šoka sa znacima multiorganskog zatajenja, doneta je odluka da se započne sa venoarterijskom ekstrakorporalnom membranskom oksigenacijom. Neposredno nakon započinjanja vantelesne membranske oksigenacije, uspostavljena je diureza. Tokom narednih 36 časova postignuta je adekvatna perfuzija organa sa kompletnim povlačenjem znakova multiorganskog zatajenja. Nakon ponovnog uspostavljanja funkcije svih organa, bolesnica je podvrgnuta de-

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kanulaciji nakon čega je, desetog dana, otpuštena iz bolnice u dobrom stanju. **Zaključak.** U slučajevima refraktornog kardiogenog i distributivnog šoka usled trovanja diltiazemom i ostalim agensima, venoarterijska ekstrakorporalna membranska oksigenacija može pružiti dodatnu cirkulatornu podršku i omogućiti dodatno vreme za endogeno uklanjanje toksina. Venoarterijska ekstrakorporalna membranska oksigenacija bi trebalo da se koristi zajedno sa optimalnom medikamentnom terapijom u cilju uspostavljanja perfuzije krajnjih organa čime se podstiče metabolizam lekova i toksina i njihova prirodna eliminacija.

**Ključne reči:**
**kalcijum, blokatori; oksigenacija; ekstrakorporalna, membranska; trovanje; pluća, edem; šok, kardiogeni; lečenje, ishod.**

## Introduction

Cardiovascular drugs are one of the most frequent substance category involved in human exposures/intoxications, according to the American Poison Control Centers' National Poison Data System [1]. Among cardiovascular drugs, single most frequent intoxicating agents are calcium channel blockers (CCB) and beta blockers (BB). Multidrug intoxication may potentially lead to more deleterious effects resulting in higher mortality rate [2], but is less common than single agent intoxication [1]. Trend of household detergents and germicides intoxication is on increase as well. Impact of mixing several intoxicating agents, namely cardiovascular drugs and household detergents containing anionic surfactants, remains largely unknown [3].

Diltiazem and verapamil are among the most commonly used drugs for accidental, or suicidal overdose [3, 4]. Diltiazem is associated with negative inotropic and chronotropic effect, often coupled with extensive peripheral vasodilatation effects contributing to shock development. Knowing that diltiazem is designed and produced as sustained release medication, might explain delayed onset and prolonged duration of toxicity [5]. Additional surfactant intoxication can contribute to deterioration of clinical course. Surfactant composition varies among products. Surfactants are frequently encountered in human environment in the form of household cleaning products.

The application of the concept of extracorporeal life support (ECLS) in drug induced cardiogenic shock is possible, but clinical experience is still limited with inadequate evidence to support a high-grade recommendation.

## Case report

A 36-year-old female was admitted to the Intensive Coronary Care Unit (ICCU) due to ingestion of large amount of tablets in a suicidal attempt. The patient's relatives reported ingestion of near 90 tablets of diltiazem (90 mg) – total ingested dose of 8.1 g, 50 tablets of metoprolol (100 mg) and 4 pieces of toilet refreshment agent (Bref® - Henkel).

During the admission, the patient was unconscious – Glasgow Coma Scale 9 (Eye-2, Verbal-3, Motor-4), cyanotic, tachypnoic (respiratory rate 30/min), hypotensive (blood pressure 65/40 mmHg), with a heart rate of 45 beats per min. Initial electrocardiogram (ECG) revealed atrioventricular (AV) conduction abnormalities with second (Figure 1) and third degree AV block. The standardized scale for grading severity of poisoning – Poisoning Severity Score (PSS) [6] – yielded grade 3, designating poisoning affecting more organ systems: cardiovascular, respiratory and central nervous system. Laboratory findings revealed complex abnormalities. The arterial blood gas analyses showed severe lactate acidosis [pH 7.14, normal range (nr) 7.35–7.45, lactate 9.2 (nr less than 1.0), base excess (BE) -18.6 mmol/L (nr: -3 mmol/L to +3 mmol/L)], sodium of 118 mmol/L (nr 135–145 mmol/L), potassium of 2.6 mmol/L (nr 3.6–5.2 mmol/L), and ionized calcium of 0.8 mmol/L (nr 1.0–1.2 mmol/L)]. A glucose level at admission was 16 mmol/L (nr 3.9–5.5 mmol/L).

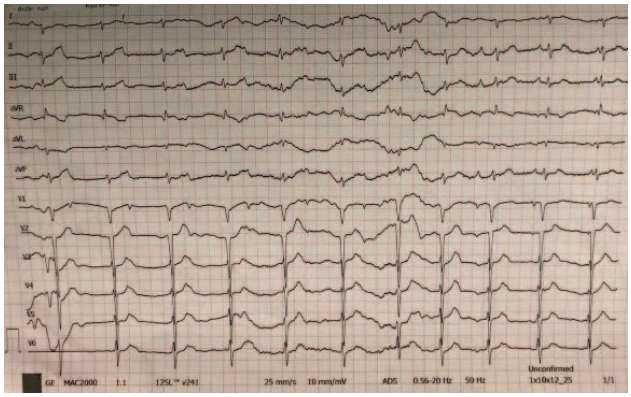

**Fig. 1 – Second-degree atrioventricular block – Mobitz 2.**

Immediately after the admission, the patient was endotrachealy intubated and mechanical ventilatory support was instituted. Following the nasogastric tube insertion and initial elimination of 500 mL of gastric content, additional repeated gastric lavage was performed, eliminating some particles of undigested tablets. Knowing that the patient ingested anionic surfactant, a decision was reached not to use additional charcoal through the nasogastric tube.

Volume resuscitation (total amount of 1.5 L) with saline infusions was started during the first 2 h. Due to a lack of prompt response, and persistent hypotension and bradycardia, dopamine (10–20 μg/kg/min) and noradrenaline (0.2–2.0 μg/kg/min) were administered. Sodium bicarbonate in total amount of 25 mmol during the first 10 h was also administered along with 20 mmol of calcium gluconate.

Chest X-ray was performed indicating the presence of pulmonary edema (Figure 2). A computed tomography (CT) scan confirmed a massive amount of undigested tablets in the patient's stomach (Figure 3), confirming the diagnosis of pulmonary edema with bilateral pleural effusion. Echocardiography revealed slightly depressed left ventricular systolic function. Summarizing all the data gained through various diagnostic modalities and clinical presentation, a diagnosis of cardiogenic and distributive shock with acute multiorgan failure was established.

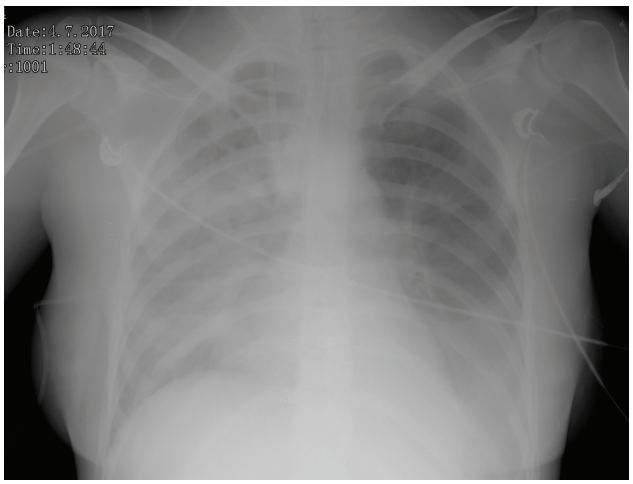

**Fig. 2 – Chest X-ray demonstrating pulmonary edema.**

The blood samples taken immediately after the admission of patient to the ICCU (approximately 7 h after ingestion) were sent to a reference toxicology laboratory for a detailed quantitative analysis. The diltiazem blood level was 6,200 ng/mL (therapeutic range 50–200 ng/mL). No traces of BB or its metabolite were found. Thus, BB intoxication was excluded. There were no possibilities to confirm anionic surfactants poisoning due to lack of reagents in laboratory.

The initial treatment strategy with inotropic and vasopressor agents was unsuccessful. The signs of progressive and refractory cardiogenic and distributive shock were apparent. The National Poison Control Center (Military Medical Academy in Belgrade) was contacted for further assistance. Additional treatment with lipid emulsion therapy (LET) was proposed. Unfortunately, LET was not available at the time, and it could not have been ordered and delivered in acceptable period.

Eight hours after continuous conservative treatment with slight, or no improvement in hemodynamic and general state of the patient, decision was made to put the patient on venoarteriral extracorporeal membrane oxigenator (VA-ECMO). A percutaneous cannulation of the left superficial femoral artery was achieved with 17 French arterial cannula with a distal perfusion protection using 7 French arterial sheet. Simultaneously, the percutaneous right femoral vein cannulation using 19 French cannula was performed (Figure 4). ECMO flow was set to around 3 L/min with a pump speed set to 3,000 rounds per minute. Within minutes, hemodynamic stability was achieved and spontaneous diuresis appeared. Initial lactate level of 9.2 mmol/L was reduced to 0.97 mmol/L in matter of 15 h following the ECMO commencement.

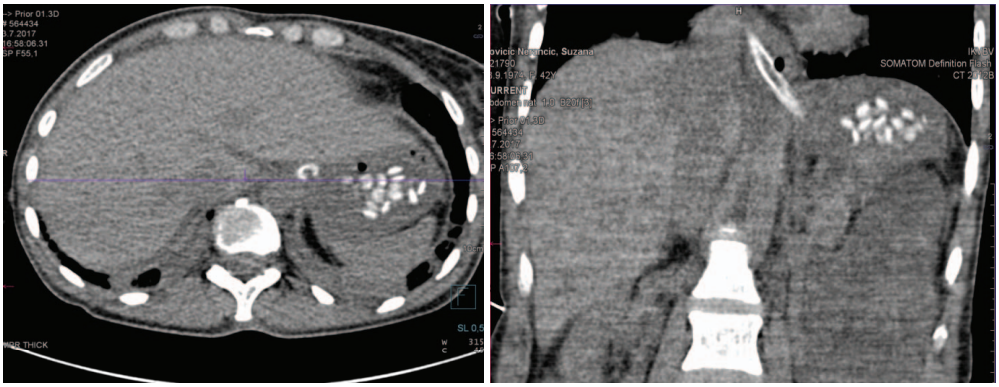

**Fig. 3 – Computed tomography (CT) scan of the abdomen showing massive amount of undigested tablets in the stomach.**

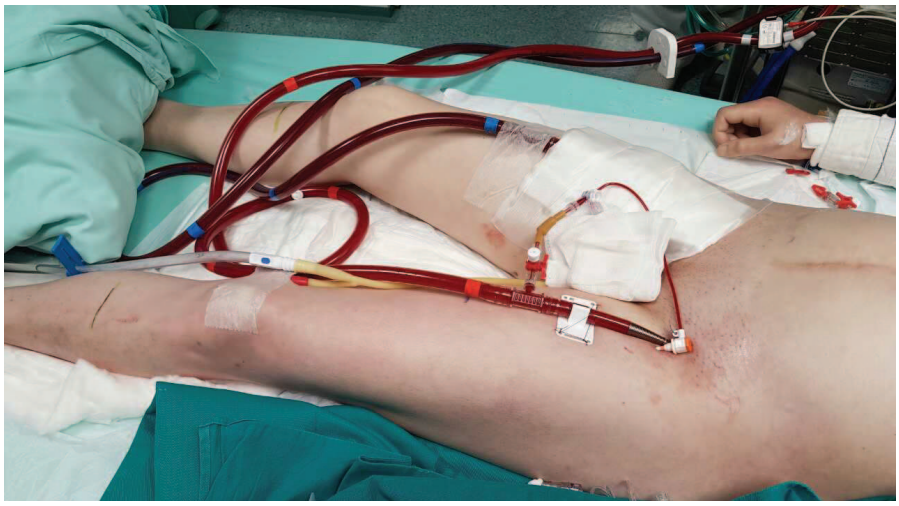

**Fig. 4 – Venoarterial extracorporeal membrane oxygenator (VA-ECMO) – the left superficial femoral artery with distal perfusion protection and the right femoral vein**

During the following 36 h, a gradual hemodynamic stabilization was established allowing successful weaning (gradual reduction of ECMO flow to 0.5 L/min with sustained hemodynamic stability) and decannulation from VA-ECMO 72 h after the ECMO commencement. A full recovery of all affected organs was established. The patient was discharged from our hospital 10 days after.

## Discussion

Standard treatment of CCB and BB intoxication consists of several general measures and specific activities for sustaining normal cardiovascular function allowing the endogenous detoxification [7]. The initial treatment of symptomatic BB and CCB poisoning is supportive. This includes early airway and respiratory support. After the ingestion of CCB, it is important to determine formulation of drugs – whether it is a slow- release, or intermediate-release drug formulation [4]. Gastrointestinal decontamination is considered one of the first procedures needed to be performed. Administration of activated charcoal within 4 hours of CCB ingestion is suggested, especially in the case of immediate release CCB formulation ingestion. Whole bowel irrigation (WBI) with a polyethylene glycol electrolyte mixtures recommended in the case of slow-release CCB ingestion and should be considered in all patients. Activated charcoal administration is contraindicated after the ingestion of corrosive substances (e.g., inorganic acids), surfactants, or liquid hydrocarbons, and whenever the respiratory tract has not been protected by intubation [5].

Specific measures suggested for a treatment of CCB poisoning include administration of high dose of insulin, calcium, dopamine, noradrenaline or LET [7–10]. As demonstrated in our case, most of the recommended therapeutic options were performed with no satisfactory result, prompting another approach – extracorporeal life support. Although VA-ECMO was used in this clinical scenario with a clear survival benefit, no large scale studies have been performed examining the efficacy of ECMO in the setting of drug induced cardiogenic and distributive shock [11–13]. In the observational study published by Masson et al. [8] ECMO support was associated with a lower mortality when initiated in a group of 14 patients compared to conventional therapies provided to a group of 48 patients (48% vs. 86%) after adjustment for the Simplified Acute Physiology Score (SAPS) II and BB intoxication. Most human case series reported the positive functional outcomes in the majority of survivors with acceptable rate of procedure- related complications.

Lange et al. [14] performed a search of several scientific databases using the keywords: “extracorporeal membrane oxygenation”, “extracorporeal life support”, “ECMO”, “ECLS”, “assist-device”, and “intox*” or “poison*”. A total of 46 publications were selected (case reports and case series) and thoroughly examined. The authors concluded that ECLS could be safely used as a bridge-to-recovery for severely intoxicated patients with cardiogenic shock. They also defined main contraindications: absolute – uncontrolled coagulopathy and severe intracranial bleeding; relative – advanced age, severe irreversible brain injury, untreatable metastatic cancer, severe organ dysfunction and high positive pressure ventilation for more than 7 days. Most commonly observed complications of ECMO are the cannulation site bleeding and intracranial bleeding.

Recently published paper from Lyon group [15] looked at the high-rate arterial complications supported by ECLS for drug induced cardiogenic shock. In the period 2010–2015 they performed 12 ECLS. Drug intoxication was mainly due to BB and/or CCB (83.3%) and 5 (41.7%) patients had multiple drugs overdose. A success rate – hospital discharge and no major neurological sequel – was 75% (9 patients) with the mean support time of 2.4 ± 1.1 days. It is interesting that 6 (50%) patients developed lower limb ischemia that had to be dealt with decannulation and other vascular procedures. For this reason, we advocate routine use of distal perfusion protection with 7 French arterial sheet.

An interesting observation came from Lee et al. [16] about the adverse effects associated with the combined use of intravenous LET and ECMO. They were able to identify 7 papers in which simultaneous use of LET and VA-ECMO was described. There is an evidence that such a combined therapy is linked with the higher rate of fat depositions in the VA-ECMO circuits and increased blood clot formation warranting increased awareness of the managing personnel. In our patient, we also engaged combined LET and VA-ECMO approach with no adverse effect that we contribute to short ECLS time, only 72 hours. Kolcz et al. [17] proposed a way to reduce emergency ECLS time through initiating therapeutic plasma exchange. This approach allowed them to circulatory support the patient while performing exogenous clearance of plasma.

## Conclusion

In severe cases of refractory cardiogenic and distributive shock due to diltiazem and other poison intoxication, VA-ECMO could give additional support and provide the bonus time for endogenous clearance of toxins. ECLS could be used in conjunction with the optimal medical therapy aiming to restoration of end-organ perfusion and allowing for intrinsic drug and toxin metabolism and natural elimination. An increasing body of evidence is accumulating for the use of ECLS in drug-induced cardiogenic shock, but further rigorous clinical trials and strong evidence are warranted.

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Received on September 26, 2017.
Revised on October 22, 2017.
Accepted on October 24, 2017.
Online First November, 2017.

CASE REPORT


UDC: 616.155.392
https://doi.org/10.2298/VSP170824174D

# Isochromosome der(17)(q10)t(15;17) in acute promyelocytic leukemia resulting in an additional copy of the RARA-PML and loss of one p53 gene: report of two cases and literature review

## Izohromozom der(17)(q10)t(15;17) u akutnoj promijelocitnoj leukemiji rezultira dodatnom kopijom RARA-PML i gubitkom p53 gena: prikaz dva slučaja i pregled literature

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**Abstract**

**Introduction.** The isochromosome of the long arm of derivative chromosome 17, that originates from the translocation t(15;17) [ider(17)(q10)t(15;17), or ider(17q)] in acute promyelocytic leukemia (APL), is a rare chromosome aberration associated with a poor prognosis. **Case report.** We report the clinical and laboratory data associated with ider(17q) for two APL patients. Cytogenetic analysis of bone marrow cells in both cases showed a mosaic karyotype with the ider(17q); reverse transcription polymerase chain reaction (RT-PCR) was positive for the long (L) isoform of the retionic acid receptor alpha (PML-RARA) fusion transcript in each patient. Fluorescence *in situ* hybridization (FISH) analysis with the DNA probes for the PML gene on 15q24.1, and the RARA gene on 17q21.2, confirmed the extra copy of the RARA-PML fusion gene or ider(17q). Additionally, the FISH analysis with a DNA probe for the p53 gene on 17p13.1 confirmed loss of one copy of the universal tumor suppressor p53 in both patients. **Conclusion.** Both reported APL patients with ider(17q) had predominance of the clone with ider(17q) compared to those with t(15;17) and/or the normal karyotype, indicating that duplication of der(17) may provide a growth advantage allowing the relevant clone to become dominant. Moreover, as an important oncogenic event and poor prognostic factor in leukemia, loss of one gene copy of the tumor suppressor p53, may also contribute to this growth advantage. Although the clinical and prognostic significance for the patients with an ider(17q) remains unclear, cytogenetic and molecular-genetic analysis should be combined to reveal more details about this complex and rare chromosomal abnormality.

**Key words:**
**leukemia, myeloid; chromosome abberations; transcription factors; in situ hybridization, fluorescence; translocation, genetic; genes, p53; mortality; retinoic acid receptor alpha.**

**Apstrakt**

**Uvod.** Izohromozom dugog kraka derivata hromozoma 17, koji potiče od translokacije t(15;17) [ider(17)(q10)t(15;17) ili ider(17q)] u akutnoj promijelocitnoj leukemiji (APL), je retka hromozomska aberacija, povezana sa lošom prognozom. **Prikaz bolesnika.** Prikazali smo dva bolesnika čiji su klinički i laboratorijski podaci ukazivali na dijagnozu APL. Citogenetička analiza ćelija kostne srži oba bolesnika, pokazala je mozaičan kariotip sa ider(17q); reverzna transkripcija lančane reakcije polimeraze (RT-PCR) bila je pozitivna za dugu (L) izoformu fuzionog PML-RARA (retinoinska kiselina receptor alfa) transkripta. Analiza, fluorescentna *in situ* hibridizacija (FISH) sa DNA probama za PML gen na 15q24.1 i RARA gen na 17q21.2, potvrdila je prisustvo dodatne kopije RARA-PML fuzionog gena ili ider(17q). Pored toga, FISH analiza sa DNA probom za p53 gen na 17p13.1 potvrdila je gubitak jedne kopije univerzalnog tumor supresor gena p53 kod oba bolesnika. **Zaključak.** Kod oba bolesnika registrovana je dominacija patološkog klona sa ider(17q) u odnosu na klon sa klasičnom translokacijom t(15;17) i/ili klon sa normalnim kariotipom, što ukazuje na mogućnost da duplicirani der(17q) obezbeđuje proliferativnu prednost patološkom ćelijskom klonu. Nastankom ove aberacije dolazi i do gubitaka jedne kopije univerzalnog tumor supresor gena p53. To je dodatni, onkogeni događaj u neoplastičnom pro-

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cesu i loš prognostički parameter za bolesnike sa APL, jer se time povećava efekat proliferativne prednosti leukemijskih ćelija. Iako klinički i prognostički značaj aberacije ider(17q) i dalje ostaje nejasan, kroz primer dva naša bolesnika možemo zaključiti da se isključivo kombinovanjem citogenetičkih i molekularno-genetičkih analiza mogu donekle obezbediti uslovi za razotkrivanje detalja o ovoj kompleksnoj i retkoj hromozomskoj abnormalnosti.

**Ključne reči:**
**leukemija, mijelocitna, akutna; hromosomi, aberacije; faktori transkripcije; hibridizacija in situ, fluorescentna; translokacija (genetika); geni p53; mortalitet; retinoična kiselina, receptor alfa.**

## Introduction

Acute promyelocytic leukemia (APL) is a well-defined clinical and biological entity in acute myeloid leukemias, characterized by unique morphology of leukemic cells and specific t(15;17), present in approximately 80% of APL cases [1, 2]. The t(15;17)(q22;q21) fuses the PML (promyelocytic leukemia) gene on chromosome 15 encoding a transcription factor with the RARA (retinoic acid receptor alpha) gene located on chromosome 17, a member of a steroid hormone nuclear receptor family that is important for regulation of both normal and malignant cellular differentiation and proliferation [3]. The ider(17)(q10)t(15;17)(q22;q21) is an infrequent variant of cytogenetic abnormality among the APL patients, which has been rarely reported. It is considered that ider(17q) is an additional chromosome aberration to t(15;17) and the second step in leukemogenesis in APL. Additional chromosome aberrations to t(15;17) have been observed in 23%–43% of APL cases, but their prognostic significance is still controversial [4–9]. The majority of evidence supports the concept that the patients with additional chromosome abnormalities have the same favorable prognosis as the patients with t(15;17) alone [5, 8]. However, some reports described a worse outcome both in newly diagnosed and relapsed patients [6, 7]. The most frequent secondary aberration to t(15;17) is trisomy 8 (+8). Other additional chromosome changes include del(9q), del(7q), abnormalities of chromosome 1, 3 and 6, trisomy 21, as well as an isochromosome of the long arm of the derivative chromosome 17 originating from the translocation t(15;17) [ider(17)(q10)t(15;17) or ider(17q)] [9]. However, the clinical significance of ider(17q) has not been elucidated yet. The clinical features and prognosis of patients with this chromosomal abnormality are currently unclear [4, 10]. To our knowledge, 74 APL cases associated with ider(17q) have been described to date [2–5, 10–22].

Here, we describe two cases of adult APL with ider(17)(q10), identified by conventional cytogenetics, fluorescence *in situ* hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR). We discuss the clinical course and follow-up data of these patients. Also, we present a combination of cytogenetic and molecular-genetic analyses which indicate that ider(17q) may be critical in providing a proliferative advantage and driving clonal evolution to overt hematologic neoplasia.

## Case report

### *Cytogenetic analysis and fluorescence in situ hybridization*

The bone marrow cells were subjected to a cytogenetic analysis by a direct preparation after 24 hours culture (RPMI 1640 medium supplemented with 25% fetal calf serum, at 37ºC). Chromosomes were stained by the modified Giemsa HG-banding technique, as previously described [23]. The karyotypes were reported in accordance with the Guidelines of the International System for Human Cytogenetic Nomenclature (ISCN) [24].

Interphase and metaphase fluorescence *in situ* hybridization (FISH) studies were performed on the bone marrow cytogenetic specimens previously used for the karyotype analysis. The PML-RARA and RARA-PML fusion genes were detected using the DF SureFISH® 15q24.1 probe to label PML together with the DF SureFISH® 17q21.2 probe to label RARA (Agilent Technologies®, Cedar Creek, TX, USA). The p53 gene was detected using the LSI TP53® (17p13.1) probe (Vysis®, Downers Grove, Ill., USA). The DNA probes were applied according to standard procedures recommended by the manufacturer. The slides were examined on an Olympus® BX51 fluorescence microscope. The Dual-color FISH images were digitally generated using the CytoVision® 4.02 imaging software (Leica Biosystems®).

### *Molecular genetics*

The RT-PCR assay was employed to detect the PML-RARA fusion gene. Total RNA was extracted from the bone marrow cells and then reverse transcribed to cDNA with oligo(dT) primers. For determination of the PML/RAR-α transcript we applied a standardized RT-PCR method [25]. This enabled us to detect the most common PML/RAR-α transcripts due to the right combination of primers (Table 1), both in the first and second (nested) PCR cycles. The PCR products were separated by electrophoresis on 2% agarose gel and visualized with ethidium bromide.

### *Case 1*

A 64-year-old female patient was referred with bleeding gums and bruises on her lower extremities. The hematological work-up revealed anemia (hemoglobin 85 g/L), a very low platelet count ($10 \times 10^9$/L) and leucopenia ($2.2 \times 10^9$/L), with 10% blasts and 36% promyelocytes. The coagulation tests showed: normal fibrinogen level (3.2 g/L), decreased prothrombin time (PT 58%), normal activated partial thromboplastin time (PTT 26 s) and elevated D-dimer (31 mg/L). The calculated International Society of Thrombosis and Hemostasis disseminated intravscular coagulopathy (ISTH DIC) score was 6. A bone marrow biopsy revealed a hypercellular marrow with abundant promyelocytes (80%).

**Table 1**

**Primers for RT-PCR analysis of the PML-RARA fusion gene**

| RT-PCR | Primer code | Sequence (5'-3') |
|---|---|---|
| First round of PCR | PML-A1 (forward) | CAGTGTACGCCTTCTCCATCA |
| | PML-A2 (forward) | CTGCTGGAGGCTGTGGAC |
| | RARA-B (reverse) | GCTTGTAGATGCGGGGTAGA |
| Second round of PCR | PML-C1 (forward) | TCAAGATGGAGTCTGAGGAGG |
| | PML-C2 (forward) | AGCGCGACTACGAGGAGAT |
| | RARA-D (reverse) | CTGCTGCTCTGGGTCTCAAT |

**RT-PCR – reverse transcription-polymerase chain reaction; PML – promyelocytic leukemia; RARA – retinoid acid receptor alpha.**

Immunophenotyping of leukemia cells from the bone marrow also confirmed predomination of promyelocytes with a typical immunophenotype: $CD117^{+low}$ $CD13^{+hetero}$ $CD33^{+high}$ $cMPO^{+high}$ $CD15^{+hetero}$ $CD34^{neg}$ HLA-$DR^{neg}$ $CD11a^{neg}$ $CD11b^{neg}$ $CD56^{neg}$ $CD2^{neg}$. The patient was treated according to the PETHEMA 2005 regimen (all-trans retinoic acid – ATRA and idarubicine). During the induction therapy, she experienced forehead necrosis in a previous hematoma. After necrectomy with reconstruction, the patient successfully completed the induction and with intensified treatment according to the same PETHEMA protocol achieved complete remission. She also completed the maintenance schedule and is still in complete remission with a good health status.

*Case 2*

A 58-year-old man was admitted to the Clinic of Hematology, Clinical Centre of Serbia, Belgrade, with breathlessness, weight loss, night sweats and fever. Physical findings revealed numerous bruises and hematuria. His blood counts revealed severe pancytopenia with hemoglobin at 106 g/L, a white blood cell count of $1.7 \times 10^9$/L, with 24% undifferentiated blasts and a low platelet count of $17 \times 10^9$/L. The hemostasis testing also showed prolonged PT (61%), but normal PTT. D-dimer was highly elevated, but a fibrinogen concentration was preserved (6.9 g/L). The ISTH DIC score was also elevated to > 6. The bone marrow examination revealed 84% of blasts with bilobar nuclei and multiple Auer rods ("faggots"). Immunophenotyping confirmed the presence of a pathological population of promyelocytes with a typical immunophenotype: $CD117^{+low}$ $CD13^{+low}$ $CD33^{+low}$ $cMPO^{+high}$ $CD15^{neg}$ $CD34^{neg}$ HLA-$DR^{neg}$ $CD11a^{neg}$ $CD11b^{neg}$ $CD56^{neg}$ $CD2^{neg}$, corresponding to APL. The patient was also treated according to the PETHEMA 2005 regimen, but unfortunately a lethal outcome occurred within several days due to the acute respiratory distress syndrome together with a deterioration of DIC and bleeding in spite of supportive measures.

*Cytogenetics*

Cytogenetic analysis in both patients revealed the chromosome 17 aberration, ider(17)(q10). The translocation t(15;17), as the primary aberration, was detected in one case. The cytogenetic results are presented in Table 2 and the karyotypes of the cell clones with ider(17q) are shown in Figures 1 and 2.

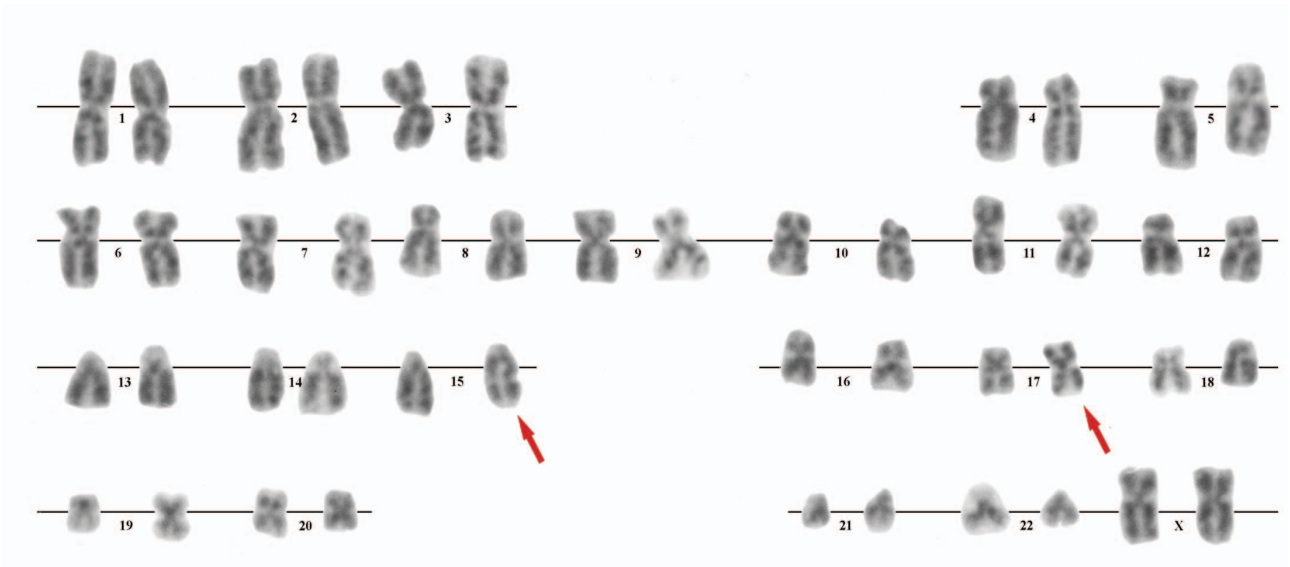


**Fig. 1 – Giemsa HG–banded bone marrow karyotype of the Case 1 showing 46,XX,der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q21). The arrows indicate abnormal chromosomes.**

**Table 2**

**Results of cytogenetic, FISH and RT-PCR analyses of bone marrow cells of the patients with ider(17q)**

| Case No. | Sex/ age | Karyotype | FISH* | | | | RT-PCR |
|---|---|---|---|---|---|---|---|
| | | | Probes | Clone (%) | Probe | Clone (%) | PML-RARA |
| | F/M | | DF SureFISH 17q21.2 and 15q24.1 | | LSI TP53 17p13.1 | | isoforms (S, V, L) † |
| 1 | F/64 | 46,XX,der(15)t(15;17)(q22;q21), ider(17)(q10)t(15;17)(q22;q21)[5]/ 46,XX[15] | nucish(PML,RARA)x4 (RARAconPMLx3)[86/200], (PML,RARA)x3 (RARAconPMLx2)[64/200], (PML,RARA)x2[20/200] | 43/32/25 | nucish(p53x1)[81/200], (p53x2)[119/200] | 41/59 | L |
| 2 | M/58 | 46,XY,der(15)t(15;17)(q22;q21), ider(17)(q10)t(15;17)(q22;q21)[10]/ 46,XY,t(15;17)(q22;21)[8]/46,XY[2] | nucish(PML,RARA)x4 (RARAconPMLx3)[69/200] (PML,RARA)x3 RARAconPMLx2)[101/200], (PML,RARA)x2[33/200] | 35/51/17 | nucish(p53x1)[81/200], (p53x2)[119/200] | 38/62 | L |

***FISH signals counting of 200 interphase nuclei. The cut-off value for positivity was the presence of more than 10% of nuclei for fusion signals of PML-RARA and for *p53* monosomy.**

**†S, V, L -short, variant, long isoforms of *PML-RARA***

**FISH – Fluorescence *in situ* hybridization; PML-RARA – promyelocytic leukemia-retinoic acid receptor alpha.**

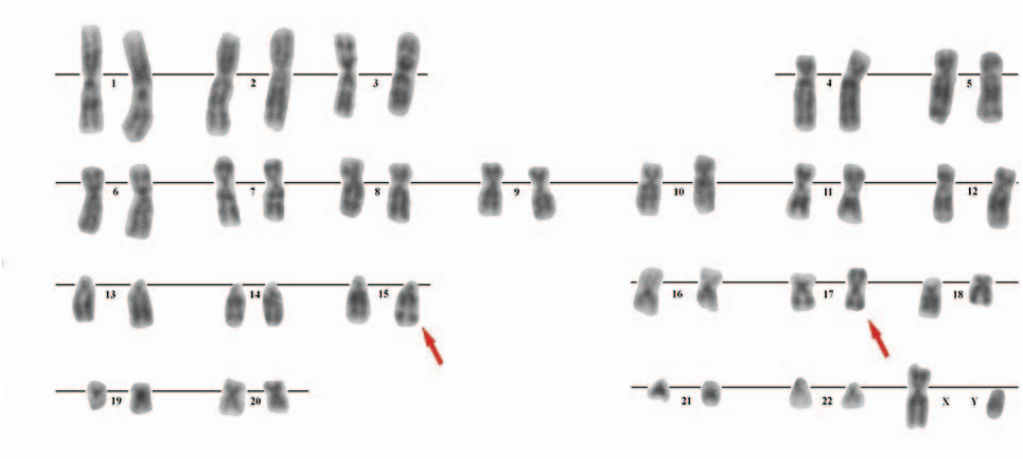

**Fig. 2 – Giemsa HG–banded bone marrow karyotype of the Case 2 showing 46,XX,der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q21). The arrows indicate abnormal chromosomes.**

The banding studies suggested that the aberrant chromosome resulted from duplication of the long arm of chromosome 17 or duplication of der(17)(10)t(15;17). The newly created chromosome looked like a classical i(17)(q10). Therefore, it was necessary to apply the metaphase and interphase FISH and RT-PCR analysis to determine whether the *PML-RARA* gene fusion is present on the i(17q).

*Fluorescence in situ hybridization*

Using the DNA probes for the PML and RARA genes, we detected the typical fusion pattern as well as the variant fusion pattern in the metaphase and interphase cells. The variant fusion pattern with one fusion gene for PML-RARA and two for RARA-PML corresponded to the clone with ider(17q), while the typical fusion pattern with one fusion for PML-RARA and one fusion for RARA-PML corresponded to the clone with t(15;17). Using a DNA probe for the p53 gene on 17p13.1 we registered loss of one copy of this normal tumor suppressor in both patients. Furthermore, in metaphases with ider(17q), the p53 probe showed a single signal derived from the normal chromosome 17, confirming a loss of the short arm of chromosome 17 as a consequence of duplication of der(17q). The FISH results for both patients are presented in Table 2 and Figures 3 and 4.

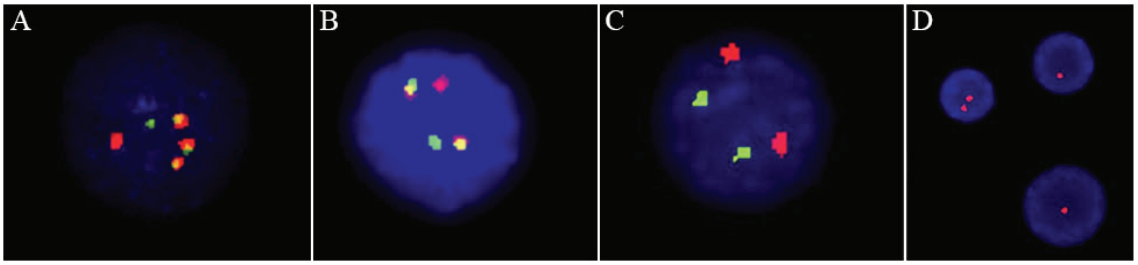


**Fig. 3 – Fluorescence *in situ* hybridization (FISH) analysis at initial diagnosis of the Case 1.**

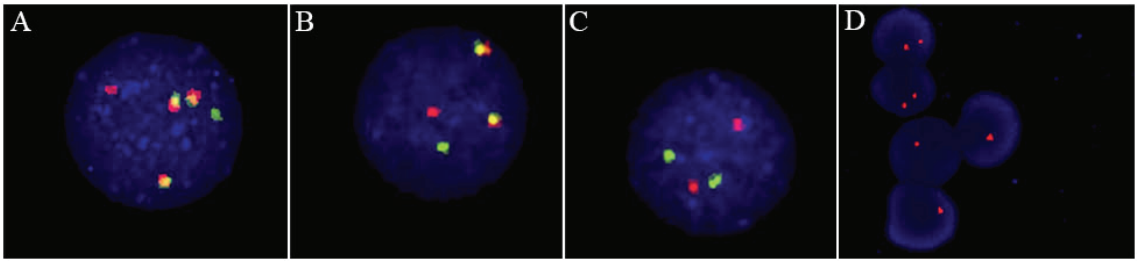


**Fig. 4 – Fluorescence *in situ* hybridization (FISH) analysis at initial diagnosis of the Case 2.**

**Fig. 3 and Fig. 4 – FISH study using a Promyelocytic leukemia–retinoic acid receptor alpha (PML-RARA) DF SureFISH probes (Agilent Technologies, Ceder Creek, TX, USA) and a p53 LSI TP53 probe (Vysis, Downers Grove, Ill., USA) at the initial diagnosis of both cases. (A) The interphase cell showing one PML-RARA and two RARA-PML fusion signals, one orange (PML) signal, and one green (RARA), consistent with the karyotype of ider(17q). (B) The interphase cell showing one PML-RARA, and one RARA-PML fusion signal, one orange (PML) signal, and one green (RARA) signal, consistent with the karyotype of the t(15;17). (C) The interphase cell sowing two orange (PML), and two green (RARA) signals, consistent with the normal karyotype. (D) The interphase cells showing one orange p53 signal, consistent with the karyotype of ider(17q), and two orange p53 signales, consistent with the t(15;17) or the normal karyotype.**

The FISH signals from the PML and RARA probes in case 1 indicated that 43% of the cells had the ider(17q), 32% of them were with the t(15;17), and 25% of the examined cells were normal for *PML* and *RARA* (Figure 3 A, B, C).

The FISH signals from the p53 gene indicated that 41% of the cells had lost one gene copy of p53, while 59% of them were normal (Figure 3D).

The FISH signals from the PML and RARA probes in case 2 indicated that 35% of the cells were with the ider(17q), 51% of them had the t(15;17), while 17% of them were normal for PML and RARA (Figure 4 A, B, C).

The FISH signals from the p53 gene indicated that 38% of the cells showed a loss of one gene copy of p53, while 62% were normal (Figure 4D).

*Molecular genetics*

The reverse transcription polymerase chain reaction analysis for the *PML-RARA* rearrangement in both patients gave positive results for bcr-1 (long-L isoform) (Table 2) and the diagnosis of APL was confirmed.

## Discussion

An isochromosome of the long arm of the derivative chromosome 17 is rarely observed in the APL patients [2–5, 10–22]. Thus, ider(17q) is almost always detected in an evolutionarily more advanced cell clone, as an additional chromosomal aberration [4]. Both of our cases had the typical features of APL at a diagnosis. Molecular cytogenetic analysis demonstrated an extra copy of RARA-PML as a consquence of der(17)(q10)t(15;17), indicating that two events had occurred involving the same chromosomes (15 and 17). The first results in the typical t(15;17)(q22;q21) structure with one PML signal on the normal chromosome 15, one RARA signal on the normal chromosome 17 and two fusion signals [one for PML-RARA on der(15) and one for RARA-PML on der(17)]. The second event, considered as karyotype evolution, involves duplication of the long arm of the der(17), with consequent formation of ider(17q) involving an additional copy of RARA-PML and loss of the whole short (p) arm of chromosome 17.

In one of our cases, a clone with ider(17q), but without t(15;17) was detected by the conventional cytogenetic analysis. However, the FISH analysis on interphase nuclei revealed the presence of both clones. The other patient exhibited coexistence of two pathologic clones, which were confirmed by both of cytogenetic and FISH analyses. We noticed that the clone with t(15;17) was predominant in interphase cells, while the other one with ider(17q) was more frequent in metaphases, indicating higher proliferative capacity.

The FISH analysis in both patients revealed a loss of one gene copy of the universal tumor suppressor, p53, which certainly could not be detected by the classical cytogenetic analysis. The FISH results for RARA-PML [ider(17q)] directly confirmed that ider(17q) is fully responsible for the loss of this tumor suppressor.

Our findings indicate that ider(17)(q10) might provide a proliferative and growth advantage for the leukemic clone to become dominant during the disease progression, which is in accordance with the previously published cases [2–4, 10, 11]. Unfortunately, due to the limited number of patients with ider(17q) studied by the FISH analysis, the prognostic significance of the clone size (described as the relative number of cells with ider(17)(q10)) in APL is still unclear.

The chromosomal breakpoints regions were variously mapped to regions 15q22-24 and 17q11-21 in classical translocation t(15;17) [25]. Three PML-RARA isoforms known as L-, V- and S-type transcripts are generated by breakpoints located within the bcr-1, bcr-2 and bcr-3 regions respectively, pointing to the variability in the chromosome 15 breakpoints. The most frequent isoforms are L and S (55% and 40%), while the V isoform is rare in APL (5%) [25]. Thus, ider(17)(q10) is observed in all subtypes of the PML-RARA fusion gene, but with an increased frequency of the L isoform [4]. At diagnosis, no correlations were found with respect to sex, platelet count, presence of coagulopathy or retinoic syndrome, when comparing patients with L and S-isoform PML-RARA transcripts [25].

However, Manola et al. [4] reported four adult APL cases with ider(17)(q10) and gave an extensive review of 49 previously reported APL cases with this unique chromosomal abnormality. They concluded that ider(17)(q10) was more frequent in the male than in female patients (2.12:1) with predominance of the L isoform PML-RARA fusion transcript, as well as a low initial white blood cell count. They also reported that the most frequent accompanying secondary chromosomal abnormality is trisomy 8.

Among our patients with APL, this rare finding has been seen in only two cases during the last 20 years, so we cannot speculate further about its frequency and distribution among the sexes. Leukopenia was evident in each case. Moreover, both patients had the L-isoform of the PML-RARA gene rearrangement, without additional aberrations.

In the neoplastic process generating APL, the ider(17q) bearing RARA-PML fusion represents a unique rearrangement that is a specific molecular marker for this entity.

The newest fusion RARA partner is the STAT5b gene, identified initially in a patient carrying the AML-M1 FAB entity, with a proportion of blasts exhibiting microgranular APL morphology [26]. Like the RARA gene, the STAT5b gene is localized on chromosome 17q21.1-21.2 and the two genes are estimated to be about 3Mb apart [27].

In the APL patients, the prognostic significance of ider(17q) and two copies of RARA-PML as a consequence is currently unknown. This is mainly due to the low incidence of ider(17q), as well as the sporadic limited data regarding the clinical course and outcome for the previously reported patients with ider(17q) [2–5, 10–22].

It has been known, up to now, that the PML-RARA gene, expressed in 97%-100% of all APL cases with t(15;17) at diagnosis, is involved in primary APL pathogenesis and confers sensitivity to ATRA [4, 28, 29]. However, the knowledge about the role of RARA-PML in the pathogenesis of APL is very obscure [30]. This fusion gene is expressed in 70%-80% of t(15;17) positive APL cases [4]. Expression of RARA-PML alone is sufficient for the cytological APL phenotype, but

does not confer sensitivity to ATRA [29, 30]. Furthermore, some experimental findings suggested that RARA-PML may potentiate the leukemogenesis of PML-RARA via mechanisms that are not yet understood, and therefore the exact role of RARA-PML has not been elucidated yet.

## Conclusion

In this report two patients with APL with the chromosomal abnormality ider(17)(q10) and spliced long-type PML-RARA fusion isoforms were described. The cytogenetic and FISH analysis identified karyotypes with this rare chromosomal abnormality, while RT-PCR provided additional important information about the alteration in the PML-RARA fusion gene. Prospective studies combined with cytogenetic and molecular-genetic techniques in the patients with an ider(17)(q10) may enable better understanding of the clinical, cytogenetic and molecular features, as well as the prognostic significance of APL with this chromosomal abnormality.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, N° 41004.

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Received on August 24, 2017.
Revised on November 06, 2017.
Accepted on November 07, 2017.
Online First November, 2017.

CASE REPORT


UDC: 616.517-06:616.14-005.6/.7(043)
https://doi.org/10.2298/VSP170717077M

# Psoriasis as a risk factor of pulmonary embolism – case report

## Psorijaza kao faktor rizika od plućne embolije

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**Abstract**

**Introduction.** Deep vein thrombosis and pulmonary embolism, known as venous thromboembolism, constitute a major global burden of disease. Both entities share the same risk factors. Psoriasis is a common, chronic skin disease. It also presents multisystemic inflammation, mainly affecting skin and joints, but it is also associated with the significant cardiovascular and metabolic states and comorbidities, on the so-called "psoriatic march". **Case report.** We presented a 78-year-old female patient, with psoriasis associated with pulmonary embolism which is accidentally discovered. We did not find any other predisposing factor of this disease (primary or secondary thrombophilia), except hyperhomocysteinemia. The patient was treated with low molecular weight heparin (enoxaparin), followed by the administration of an oral vitamin K antagonist (warfarin sodium) in the weight adjusted regimens. Additionally, we recommended vitamin B complex, including folate. Supposed link between hyperhomocysteinemia and psoriasis was the decreased serum folate level as the result of increased vitamin utilization in the skin because of increased DNA synthesis. **Conclusion**. The reported case reflects existing literary knowledge about the increased risk of VTE and arterial thromboembolic events in the psoriatic patients. The highest risk appears in the patients with a severe disease and may be a consequence of systemic inflammation and hyperhomocysteinemia.

**Key words:**
**venous thrombosis; pulmonary embolism; psoriasis; risk factors; comorbidity; homocysteine.**

**Apstrakt**

**Uvod.** Duboka venska tromboza i plućna embolija, poznate kao venski tromboembolizam (VTE), predstavljaju veliko globalno opterećenje. Oba entiteta dele iste faktore rizika. Psorijaza je česta hronična bolest kože. Takođe, predstavlja multisistemsko inflamatorno oboljenje, dominantno zahvatajući kožu i zglobove koje je povezano sa značajnim kardiovaskularnim, metaboličkim stanjima i komorbiditetima, tzv. "psorijatični marš". **Prikaz bolesnika.** U radu prikazujemo 78-godišnju bolesnicu sa psorijazom udruženom sa plućnim embolizmom koji je slučajno otkriven. Nije utvrđen drugi predisponirajući faktor (primarna ili sekundarna trombofilija), izuzev hiperhomocisteinemije. Bolesnica je lečena niskomolekulskim heparinom (enoksafarin) i oralnim antagonistom vitamina K (varfarin natrijum), u dozama određenim prema telesnoj težini. Dodatno smo preporučili kompleks vitamina B i folate. Pretpostavljena veza između hiperhomocisteinemije i psorijaze predstavlja snižen nivo folata u serumu, kao posledica njegove povećane potrošnje u koži, zbog povećane sinteze DNK. **Zaključak.** Prikazani slučaj ilustruje podatke iz literature da su bolesnici sa psorijazom u povišenom riziku od venskog i arterijskog tromboembolizma. Rizik je viši kod bolesnika sa teškim oblikom bolesti, što može biti posledica sistemske inflamacije i hiperhomocisteinemije.

**Ključne reči:**
**tromboza, venska; pluća, embolija; psorijaza; faktori rizika; komorbiditet; homocistein.**

### Introduction

Deep vein thrombosis (DVT) and pulmonary embolism (PE), collectively referred to as venous thromboembolism (VTE), constitute a major global burden of disease [1]. Both entities share the same risk factors. Clinical presentation ranges from asymptomatic, incidentally discovered emboli to massive embolism causing right ventricle failure and immediate death.

VTE may be "provoked" in the presence of a temporary or some reversible risk factor (such as surgery, trauma, immobilization, pregnancy, oral contraceptive use or hormone replacement therapy) within the last 6 weeks to 3 months be-

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fore diagnosis, and "unprovoked" in the absence thereof. PE may also occur in the absence of any known risk factor [2].

The proportion of patients with idiopathic or unprovoked PE was about 20% in the International Cooperative Pulmonary Embolism Registry (ICOPER) [3]. Thrombosis in the veins is triggered by venous stasis, hypercoagulability and the vessel wall inflammation or injury. These 3 underlying causes are known as the Virchow triad. All known clinical risk factors for DVT and PE have their basis in one or more elements of the triad. The most important clinically identifiable risk markers for DVT and PE are a prior history of DVT or PE, recent surgery or pregnancy, prolonged immobilization, or underlying malignancy.

Psoriasis is a common, chronic skin disease usually manifested as raised, well-demarcated, erythematous plaques with adherent silvery scales. It also presents multi-systemic inflammation, mainly affecting skin and joints, but also associated with significant cardiovascular and metabolic states and comorbidities, on the so-called "psoriatic march": insulin resistance, atherosclerosis, myocardial infarction, obesity and metabolic syndrome [4–7].

We present an old age female patient with severe psoriasis complicated with PE, which is accidentally discovered.

**Case report**

A female patient, 78 years old, was admitted to the Military Medical Academy, Clinic for Dermatology and Venereology because of generalized psoriasis. A diagnosis was established 31 years ago, since when she had been treated with topical corticosteroids and PUVA (psoralen + UVA treatment) phototherapy with temporary and partially improvement of psoriasis. A classic systemic oral therapy and biologics were never used. She denied any respiratory symptoms, except fatigue when she walked about 50 meters, during last two months. Her past medical history included stabile angina pectoris, chronic atrial fibrillation and arterial hypertension. She denied any other diseases, allergies, smoking, or alcohol consumption. A physical examination on admission revealed generalized dark red, sharply demarcated skin plaques covered with silvery scale on the trunk and extremities (Figure 1).

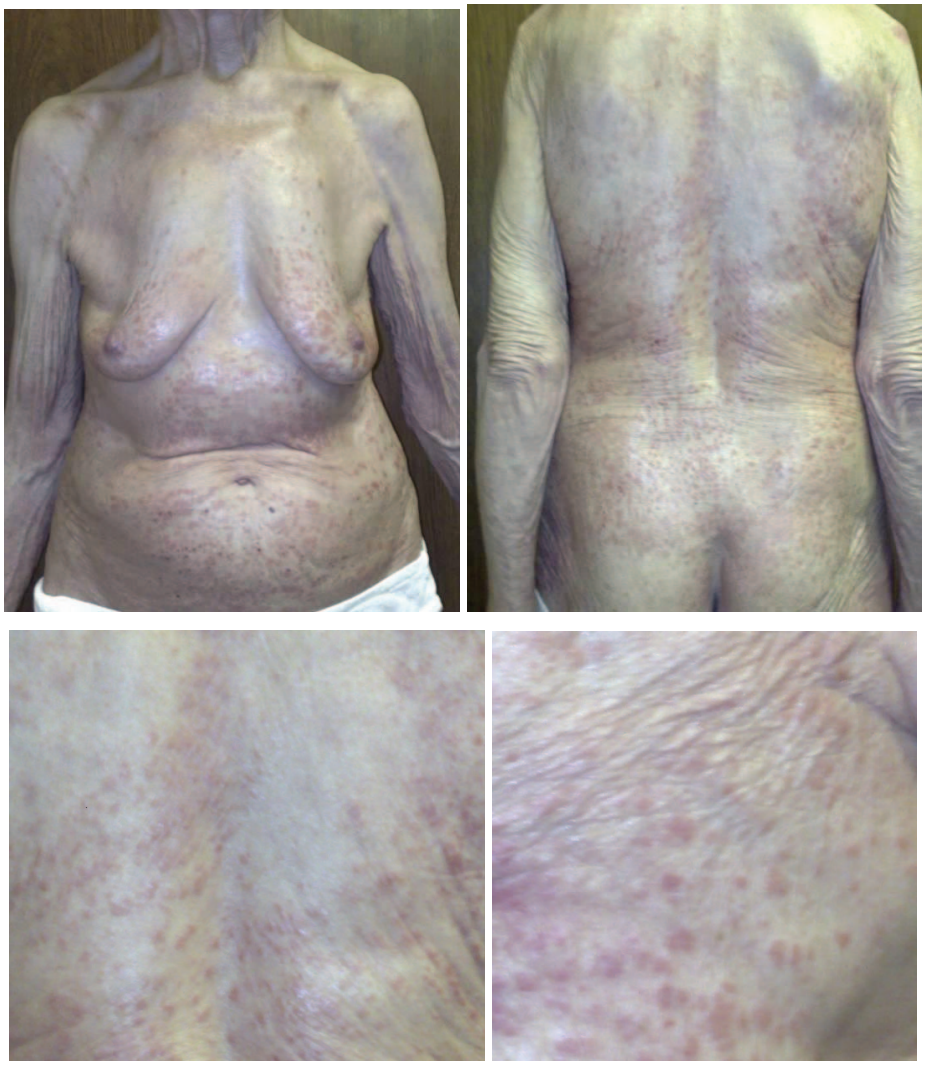

**Fig. 1 – Psoriatic skin changes: scaly erythematous plaques on trunk and extremities.**

PASI (Psoriasis Area and Severity Index) was 44. The physical finding on the respiratory system and heart was normal, except absolute arrhythmia. Also, varicose veins of lower legs were noted. Chest radiography (CXR) showed elevated right hemidiaphragm and bilateral diffuse linear and reticular pattern, predominantly in the lower lung fields. The heart's shadow was enlarged (Figure 2). Chest multidetector computed tomography (MDCT) revealed moderate fibrous changes in the lung parenchyma and bilateral embolus in the lobar and segmental branches of pulmonary artery (Figure 3).

**Fig. 2 – Chest radiography of the patient.**

The patient was moved to the Clinic for Pulomonology because of additional examination and treating. Initial laboratory tests revealed an erythrocyte sedimentation rate (ESR) of 39 mm/h (normal range 0–12), elevated C-reactive protein (CRP) – 11.39 mg/L (normal range 0–3 mg/L), normal platelets, white and red blood cells counts, decreased hemoglobin level – 94 g/L (normal range 115–165 g/L), decreased hematocrit – 0.31 (normal 0.37–0.47). The differential blood count was normal. The routine biochemical analyses (electrolytes, glucose, urea, creatinine, bilirubin, transaminases, lactate dehydrogenase, gama-glutamyl-transpeptidase, triglycerides, low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol and tumor markers (CEA, CA 15-3, CA 125, CA 19.9, CA 72.4, NSE, Cyfra 21.1) were normal. The homocystein level was elevated – 23 μmol/L (normal range 4.9–15 μmol/L), the folate level was decreased – 4.22 nmol/L (normal 7–46 μmol/L), vitamin B12 – 249 pmol/L (normal 156–672 pmol/L). Autoantibodies (antinuclear, antibodies for extractable nuclear antigens, anticardiolipin, anti-CCP (cyclic citrullinated peptide), anti-neutrophil cytoplasmic and lupus anticoagulant) were normal. Protein C, protein S and antitrombin III were in the range of predicted values. Screening for mutations of Factor V Leiden, prothrombin variant 20210A and MTHFR (methylenetetrahydrofolate reductase) showed wild type of genes.

The pulmonary function tests showed normal spirometry, carbon monoxide diffusion capacity and the respiratory arterial blood gases at rest.

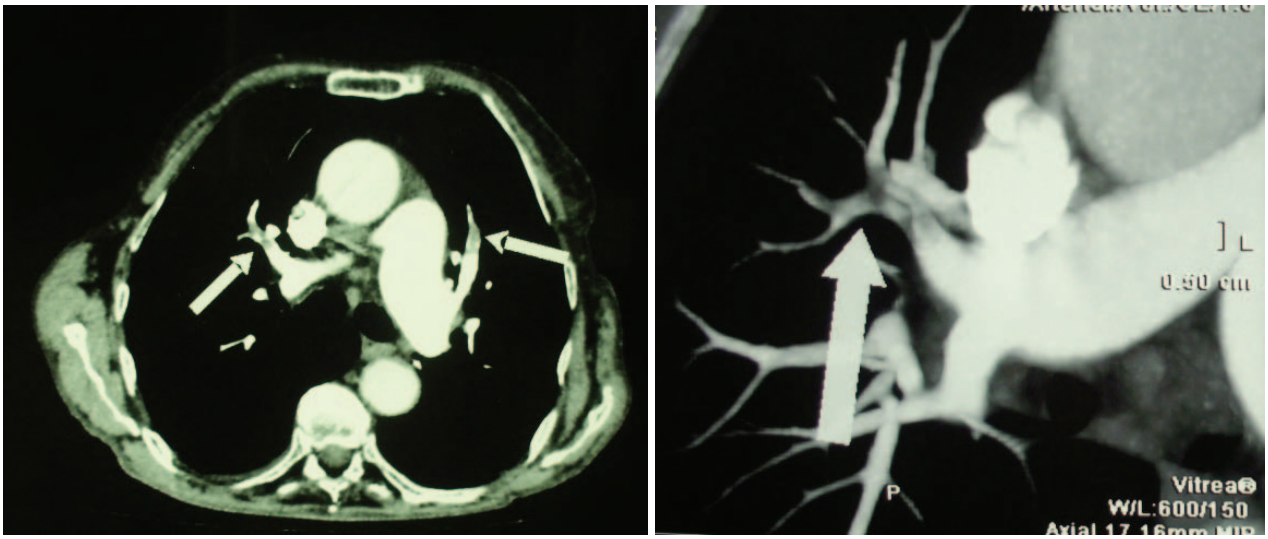


**Fig. 3 – Multidetector computed tomography (MDCT) of the same patient (emboli are marked with a white arrow).**

Electrocardiography revealed atrial fibrillation with ventricular rate 64/minute, negative T wave in D2, D3, aVF and V3-V6 precordial leads.

Doppler ultrasound of legs blood vessels showed the moderate atherosclerotic plaques in the arteries with luminal stenosis to 50% and no evidence of DVT.

Due to the fibrotic changes on the MDCT, bronchoscopy was performed. The bronchoscopic finding was normal. The bronchoalveolare lavage (BAL) fluid analysis did not show the bacterial, fungal agents, or acid fast bacilli. The BAL cytology cell profile showed macrophages 45%, lymphocytes 15% and neutrophils 40%.

The patient was treated with low molecular weight heparin (enoxaparin), followed by the administration of an oral vitamin K antagonist (warfarin sodium) in the weight adjusted regimens. Additionally, we recommended vitamin B complex, including folate.

## Discussion

Several studies have demonstrated that cardiovascular diseases and their associated risk factors are more common in the patients with psoriasis than in the general population [4–7]. The cause of this increased risk is only particularly clear. Some authors suggested that there may be some intrinsic associated risk: elevated lipids were documented in the psoriasis patients at the time of their initial diagnosis when compared to the non-psoriasis controls who were matched for the body mass index (BMI) status as well as other demographic, clinical, and lifestyle characteristics [8]. Others suggested that psoriasis was an immune inflammatory disease characterized by T helper – Th1 and Th17-driven inflammation with a striking overlap of inflammatory markers and mediators with atherosclerosis [9–11]. Also, hyperhomocysteinemia, which may be associated with atherothrombosis and VTE [11], was reported in the psoriatic patients [12–14]. The relationship between chronic inflammatory diseases (psoriasis, psoriatic arthritis and rheumatoid arthritis) was evaluated in a cohort study which was conducted in a primary care medical record database in the UK with the data from 1994–2014. The patients with mild psoriasis had the significantly elevated risks of VTE (HR 1.35, 1.29, and 1.07, respectively) after adjusting for the traditional risk factors. Severe psoriasis and psoriatic arthritis threated with disease modifying anti-rheumatic drugs, had an elevated but not a statistically significant risk for VTE. The findings were similar for DVT. The age-and-sex-adjusted risk of PE was elevated in the rheumatoid arthritis, severe psoriasis **?**and psoritac arthritis patients prescribed a disease modifying anti-rheumatic drugs [15].**?** Likewise, a Danish Nationwide Cohort Study indicated that the patients with psoriasis were at increased risk of VTE. The highest risk was found to be in the young patients with severe psoriasis [16]. In a systematic review and meta-analysis Ungprasert et al. [17] demonstrated a significant association between psoriasis and VTE with an overall 1.46-folds (95% CI 1.29–1.66) increased risk compared with the non-psoriasis participants. The risk ratios were fairly consistent across the studies, ranging from 1.35 to 1.66 [17].

In our patient, with earlier diagnosed severe psoriasis, we established PE and generalized atherosclerosis (angina pectoris and arteries in the legs). We did not find any other causes of these diseases (primary, or secondary thrombophilia), except hyperhomocysteinemia (the serum level of the homocystein was 1.5-fold elevated). Hyperhomocysteinaemia is a known risk factor for atherosclerosis and thrombosis, and may interfere with the coagulation system, causing a direct endothelial injury followed by facilitated thrombosis and causing oxidative damage to the endothelium [13]. A supposed link between hyperhomocysteinemia and psoriasis was the decreased serum folate level as the result of increased vitamin utilization in the skin because of increased DNA synthesis. Homocysteine is metabolized by either being converted into methionine or cysteine. These processes require folic acid and vitamin B12. Also, the markers of systemic inflammation (ESR, CRP) were elevated in our patient, which is in correlation with the immune-inflammatory hypothesis [9, 10].

Clinical probability of PE in our patient was low (revised Geneva score 1), so the mild fibrotic changes seen at CXR were a reason to do MDCT. Pulmonary fibrosis (PF) in the psoriatic patients usually presents a complication of systemic therapy (methotrexate, tumor necrosis factor-α inhibitors like infliximab, etanercept and adalimumab) [18, 19]. PF as an extra-cutaneous manifestation of psoriasis is uncommon [20] and usually linked to psoriatic arthritis. Anyway, we established neutrophilic alveolitis in our patient, but we did not perform the lung biopsy because of increased risk (age, comorbidities). She did not receive systemic treatment, so we did not establish etiology of PF, therefore we cannot claim that PF in our patient is a consequence of psoriasis. Several studies showed a relationship between idiopathic pulmonary fibrosis and an increased risk of vascular diseases [21, 22]. However, in our case, there was mild pulmonary fibrosis, with no radiological criteria for diffuse parenchymal (interstitial) lung diseases.

## Conclusion

The reported case reflects existing literary knowledge about an increased risk of VTE and arterial thromboembolic events in the psoriatic patients. The highest risk is in the patients with severe disease, and may be an effect of systemic inflammation and hyperhomocysteinemia.

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Received on July 17, 2017.
Revised on june 10, 2019.
Accepted July 18, 2019.
Online First September, 2019.

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c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

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*Primeri referenci:*

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**Tabele**

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

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