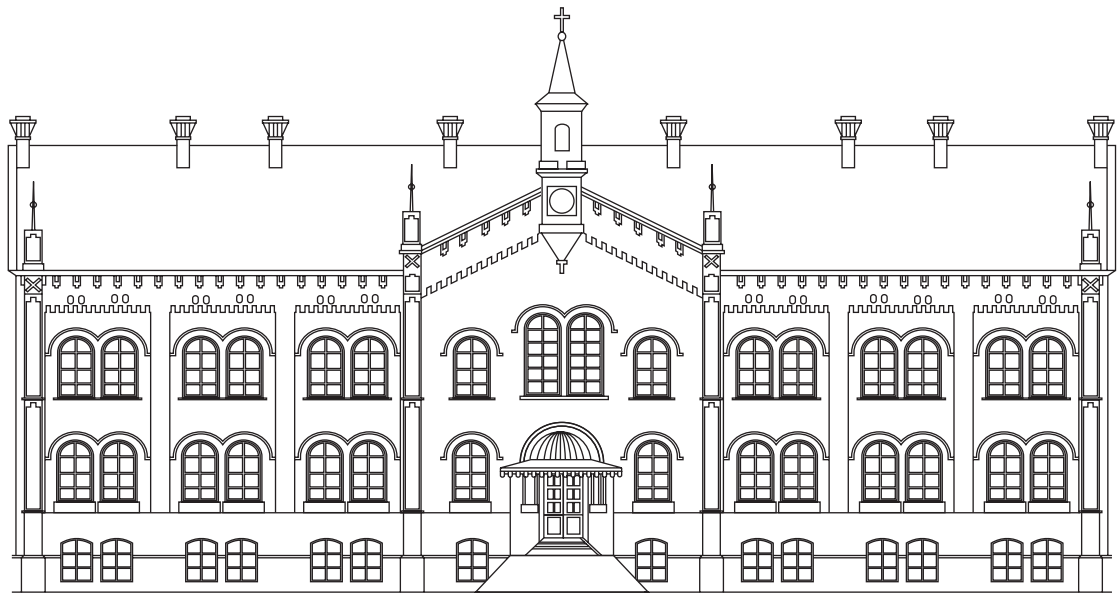
SRP ARH CELOK LEK

ISSN 0370-8179 (PRINT)
ISSN 2406-0895 (ONLINE)

COBISS.SR-ID 3378434
UDC 61(497.11)

СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО

ЧАСОПИС СРПСКОГ ЛЕКАРСКОГ ДРУШТВА

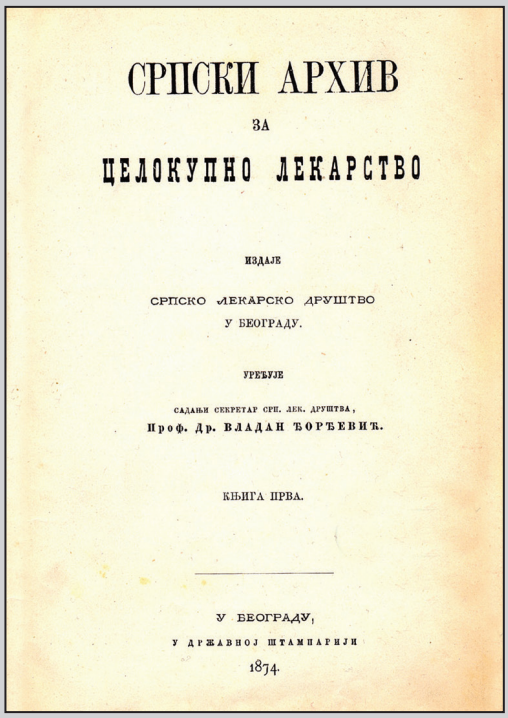


SERBIAN ARCHIVES
OF MEDICINE

JOURNAL OF THE SERBIAN MEDICAL SOCIETY

VOLUME 153 · NOVEMBER–DECEMBER 2025 · ISSUE 11–12

www.srpskiarhiv.rs



СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

САДАШЊИ СЕКРЕТАР СРП. ЛЕК. ДРУШТВА,

Проф. Др. ВЛАДАН ЂОРЂЕВИЋ.

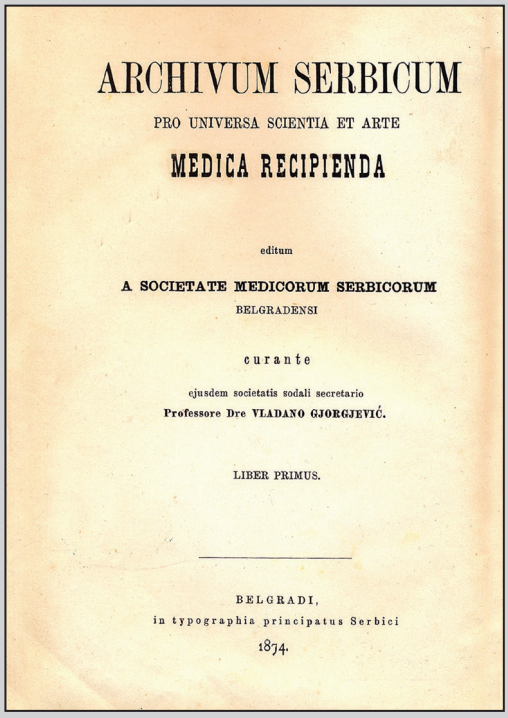
КЊИГА ПРВА.

У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



ARCHIVUM SERBICUM

PRO UNIVERSA SCIENTIA ET ARTE

MEDICA RECIPIENDA

editum

A SOCIETATE MEDICORUM SERBICORUM

BELGRADENSI

curante

ejusdem societatis sodali secretario

Professore Dre VLADANO GJORGJEVIĆ.

LIBER PRIMUS.

BELGRADI,

in typographia principatus Serbici

1874.

The title page of the first journal volume in Latin

Српски архив за целокупно лекарство је часопис Српског лекарског друштва основаног 1872. године, први пут штампан 1874. године, у којем се објављују радови чланова Српског лекарског друштва, претплатника часописа и чланова других друштава медицинских и сродних струка. Објављују се: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике и регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози.

Сви рукописи који се разматрају за штампање у „Српском архиву за целокупно лекарство“ не могу да се поднесу или да буду разматрани за публиковање на другим местима. Радови не смеју да буду претходно штампани на другим местима (делимично или у потпуности).

Приспели рукопис Уређивачки одбор шаље рецензентима ради стручне процене. Уколико рецензенти предложе измене или допуне, копија рецензије се доставља аутору с молбом да унесе тражене измене у текст рада или да аргументовано образложи своје неслагање с примедбама рецензента. Коначну одлуку о прихватању рада за штампу доноси главни и одговорни уредник.

За објављене радове се не исплаћује хонорар, а ауторска права се преносе на издавача. Рукописи и прилози се не враћају. За репродукцију или поновно објављивање неког сегмента рада публикованог у „Српском архиву“ неопходна је сагласност издавача.

Радови се штампају на енглеском језику са кратким садржајем на енглеском и српском језику (ћирилица), односно на српском језику, са кратким садржајем на српском и енглеском језику.

Аутори прихватају потпуну одговорност за тачност целокупног садржаја рукописа. Материјал публикације представља мишљење аутора и није нужно одраз мишљења Српског лекарског друштва. С обзиром на брз напредак медицинске научне области, корисници треба да независно процењују информацију пре него што је користе или се на њу ослањају. Српско лекарско друштво, уредник или Уређивачки одбор „Српског архива за целокупно лекарство“ не прихватају било какву одговорност за наводе у радовима. Рекламни материјал треба да буде у складу с етичким (медицинским) и правним стандардима. Рекламни материјал укључен у овај часопис не гарантује квалитет или вредност оглашеног производа, односно тврдње произвођача.

Поднесени рукопис подразумева да је његово публиковање одобрио одговорни ауторитет установе у којој је истраживање обављено. Издавач се неће сматрати правно одговорним у случају подношења било каквог захтева за компензацију. Треба да се наведу сви извори финансирања рада.

Srpski Arhiv Za Celokupno Lekarstvo (Serbian Archives of Medicine) is the Journal of the Serbian Medical Society founded in 1872, and with first issue published in 1874. Serbian Archives of Medicine publishes articles of the Serbian Medical Society members, subscribers, as well as members of other associations of medical and related fields. The journal publishes the following article types: editorials, original papers, preliminary and short communications, case reports, video-articles, images in clinical medicine, review articles, current topics, articles for practitioners, history of medicine articles, language of medicine articles, medical ethics (clinical ethics, publication ethics) and regulatory standards in medicine, congress and scientific meeting reports, personal view articles, invited commentaries, letters to the editor, book reviews, professional news, In memoriam and other articles.

All manuscripts under consideration in the Serbian Archives of Medicine may not be offered or be under consideration for publication elsewhere. Articles must not have been published elsewhere (in part or in full).

The submitted manuscripts are forwarded by the Editorial Board to reviewers for editing and evaluation. If the reviewers find that the manuscript needs to be modified or amended, the copy of the report is sent to the author(s), requiring of them to make necessary modifications or amendments of the text or to provide argumentative explanation of their disagreement with the suggested reviewer's remarks. The final decision on acceptance of the article for publication is made by the Editor-in-Chief.

The authors shall not be remunerated for the published articles, and they are required to assign copyright of their papers to the publisher. Manuscripts and enclosures shall not be returned to the authors. Reproduction or repeated publication of any section of the manuscript already published in the "Serbian Archives" requires the publisher's approval.

The articles are printed in the English language with an abstract both in English and Serbian, or in the Serbian language, Cyrillic alphabet, with an abstract in Serbian and English.

Authors accept full responsibility for the accuracy of all content within the manuscript. Material in the publication represents the opinions of the authors and does not necessarily reflect opinions of the Serbian Medical Society. Because of rapid advances in the medical sciences, users should independently evaluate information before using or relying on it. Serbian Medical Society, the Editor or Editorial Board of the Serbian Archives of Medicine do not accept any responsibility for the statements in the articles. Advertising material is expected to conform to ethical (medical) and legal standards. Inclusion of advertising material in this publication does not guarantee the quality or value of such product or claims made by its manufacturer.

Submission of the manuscript implies that its publication has been approved by the responsible authorities at the institution where the work has been carried out. The publisher will not be held legally responsible should be any claims for compensation. Details of all funding sources for the work should be given.

Srp Arh Celok Lek
ISSN 0370-8179
UDC 61(497.11)
COBISS.SR-ID 3378434
Српски архив за целокупно лекарство
Званичан часопис Српског лекарског друштва
Излази шест пута годишње

ГОДИШТЕ 153 НОВЕМБАР–ДЕЦЕМБАР 2025. СВЕСКА 11–12

Часопис „Српски архив за целокупно лекарство" је индексиран у базама: Science Citation Index Expanded, Journal Citation Reports/Science Edition, Web of Science, Scopus, EBSCO, Directory of Open Access Journals, DOI Serbia.

ОСНИВАЧ, ВЛАСНИК И ИЗДАВАЧ
Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
Председник
Проф. др Милан А. Недељковић
Интернет страна: http://www.sld.org.rs

ИЗДАВАЧКИ САВЕТ
Проф. др Павле Миленковић, председник
Академик Владимир Бумбаширевић
Проф. др Љубица Ђукановић
Академик Небојша Лалић
Проф. др Милица Чоловић

АДРЕСА УРЕДНИШТВА
Српски архив за целокупно лекарство
Краљице Наталије 1, 11000 Београд, Србија
Телефон: +381 (0)11 409 27 76
+381 (0)11 409 44 79
Е-пошта: office@srpskiarhiv.rs
Интернет страна: www.srpskiarhiv.rs

ПРЕТПЛАТА И ЕКСПЕДИЦИЈА
Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
Телефон: +381(0)11 3245-149
Текући рачуни: 205-8041-21 и
355-1009094-22

Чланци у целости доступни су на интернет страници: www.srpskiarhiv.rs

Цена претплате за календарску годину је 3.000,00 динара за појединце, 6.000,00 динара за установе и 100 евра за читаоце ван Србије. Цена појединачног примерка из текуће године је 600,00 динара, а свеске из претходних година 300,00 динара.

Штампање „Српског архива за целокупно лекарство" током 2025. године помогло је Министарство науке, технолошког развоја и иновација Републике Србије.

ISSN 0370-8179; ISSN Suppl 0354-2793

eISSN 2406-0895
Отворен приступ

Штампано у Србији

РЕДАКЦИЈА
Технички уредник: Јасмина Живковић
Лектор за српски језик: Дивна Продановић
Лектори за енглески језик: Мирко Рајић, Ана Којић
Корице: *MaxNova Creative*

Штампа: ЈП „Службени гласник", Београд

Тираж: 850 примерака

Srp Arh Celok Lek
ISSN 0370-8179
UDC 61(497.11)
COBISS.SR-ID 3378434
Serbian Archives of Medicine
Official Journal of the Serbian Medical Society
Published six times per year

VOLUME 153 NOVEMBER–DECEMBER 2025 ISSUE 11–12

The journal "Srpski arhiv za celokupno lekarstvo" (Serbian Archives of Medicine) is indexed in: Science Citation Index Expanded, Journal Citation Reports/Science Edition, Web of Science, Scopus, EBSCO, Directory of Open Access Journals, DOI Serbia.

EDITORIAL OFFICE
Technical editor: Jasmina Živković
Serbian language editor: Divna Prodanović
English language editors: Mirko Rajić, Ana Kojić
Cover & Logo: MaxNova Creative

Printed by: JP "Službeni glasnik", Belgrade

Circulation: 850 copies

FOUNDER, OWNER & PUBLISHER
Serbian Medical Society
President
Prof. Milan A. Nedeljković, MD, PhD

EDITORIAL OFFICE
Serbian Archives of Medicine
Kraljice Natalije 1, 11000 Belgrade, Serbia
Phone: +381 (0)11 409 27 76
+381 (0)11 409 44 79
E-mail: office@srpskiarhiv.rs
Website: www.srpskiarhiv.rs

SUBSCRIPTION AND DISTRIBUTION
Serbian Medical Society
Džordža Vašingtona 19, 11000 Belgrade
Serbia
Phone: +381(0)11 3245-149
Bank accounts: 205-8041-21 and
355-1009094-22

Full-text articles are available at website: www.srpskiarhiv.rs

Calendar year subscription prices are as follows: 3,000 dinars for individuals, 6,000 dinars for institutions, and 100 euros for readers outside Serbia. The price of a current year issue is 600 dinars, and of issues from previous years 300 dinars.

The publishing of the Serbian Archives of Medicine during 2025 is supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia.

ISSN 0370-8179; ISSN Suppl 0354-2793

eISSN 2406-0895

Printed in Serbia

САДРЖАЈ • CONTENTS

ORIGINAL ARTICLES • ОРИГИНАЛНИ РАДОВИ

DOI: https://doi.org/10.2298/SARH250807092B
UDC: 616.94-074; 616.98:578.834; 616.98:578.834

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Prognostic value of REDS, SOFA, and D-dimer in critically ill COVID-19 patients with sepsis

Dejana Bajić[1], Milica Plazačić[2,3], Andrea Mihajlović[4]

[1]University of Novi Sad, Faculty of Medicine, Department of Biochemistry, Novi Sad, Serbia;
[2]University of Novi Sad, Faculty of Medicine, Department of Pediatrics, Novi Sad, Serbia;
[3]Institute for Child and Youth Health Care of Vojvodina, Novi Sad, Serbia;
[4]University of Novi Sad, Faculty of Medicine, Department of Physiology, Novi Sad, Serbia

SUMMARY

Introduction/Objective Early identification of high-risk patients with COVID-19-associated sepsis is essential for timely intervention and optimal ICU resource allocation. This study aimed to evaluate and compare the prognostic performance of risk-stratification of emergency department sepsis (REDS) and sequential organ failure assessment (SOFA) scores, alongside D-dimer levels, in predicting 28-day mortality among critically ill patients.

Methods A retrospective analysis was conducted on 163 critically ill adult patients with confirmed COVID-19 and sepsis (Sepsis-3 criteria) admitted to the intensive care unit of a tertiary center between November 2020 and May 2022. REDS, SOFA, and D-dimer values were assessed within 24 hours of ICU admission. Predictive value was evaluated using ROC analysis, logistic regression, and χ^2 automatic interaction detector (CHAID) decision tree modeling.

Results The overall 28-day mortality rate was 65.6%. REDS (AUC = 0.690) and SOFA (AUC = 0.680) demonstrated moderate predictive ability, while D-dimer showed lower accuracy (AUC = 0.632). REDS > 2 had the highest sensitivity (80.2%), and SOFA > 4 had the highest specificity (56.1%). Both REDS and SOFA were identified as independent mortality predictors. CHAID analysis recognized REDS as the strongest discriminator, stratifying mortality risk into three distinct groups (42.9%, 66.1%, and 84.5%).

Conclusion REDS and SOFA scores provide meaningful prognostic value in patients with COVID-19-related sepsis. REDS demonstrated a slight advantage and may serve as a simple and effective bedside tool for early risk stratification in clinical practice and future viral pandemics.

Keywords: COVID-19; sepsis; REDS score; SOFA score; prognosis; mortality

INTRODUCTION

Critically ill patients with COVID-19 and sepsis remain among the most vulnerable in intensive care units (ICUs), with persistently high mortality rates despite advances in supportive care [1, 2, 3]. The convergence of viral pneumonia, immune dysregulation, and sepsis-induced multiorgan failure creates complex clinical scenarios, underscoring the urgent need for reliable early prognostic tools [4]. Timely risk stratification is essential to inform therapeutic decisions, optimize resource allocation, and improve patient outcomes. While numerous studies have explored individual biomarkers and severity scores, there is still no consensus on the most effective approach for early mortality prediction in this high-risk group [5, 6, 7]. Notably, risk-stratification of emergency department sepsis (REDS) scoring system has been shown to be a simple and objective tool for risk stratification in patients with suspected sepsis, although these studies did not include patients with COVID-19 [8].

This study provides novel insights by directly comparing the prognostic performance of three early indicators – the sequential organ failure assessment (SOFA) score, REDS score, and D-dimer levels – within the first 24 hours of ICU admission in COVID-19 patients with sepsis. Unlike prior research that typically assessed single markers or lacked rigorous validation, our study integrates multiple established tools and employs advanced statistical techniques, including bootstrap-adjusted multivariate logistic regression and χ^2 automatic interaction detector (CHAID) decision tree modeling, to enhance predictive power and internal validity [9, 10, 11].

The most notable finding is the potentially valuable prognostic role of the REDS score in predicting 28-day mortality, supported by receiver operating characteristic (ROC) analysis, multivariate models, and decision tree classification. A simplified model based on REDS thresholds effectively stratified patients into clinically relevant risk groups, supporting its utility in ICU triage and early management. The use of bootstrap validation further mitigates limitations common to retrospective studies, such as small sample size and data skewness, enhancing the robustness of our conclusions.

This study was conducted in critically ill COVID-19 patients with sepsis. While the findings provide valuable insights for this specific population, their applicability to future pandemics caused by similar viral pathogens remains to be investigated. Given the challenges

Received • Примљено: August 7, 2025
Revised • Ревизија: November 6, 2025
Accepted • Прихваћено: December 1, 2025
Online first: December 4, 2025

Correspondence to:
Dejana BAJIĆ
University of Novi Sad
Faculty of Medicine
Department of Biochemistry
Hajduk Veljkova 3
21137 Novi Sad
Serbia
dejana.bajic@mf.uns.ac.rs
dejnadeki@gmail.com

posed by comorbidities, immunosuppression, and variable vaccine responsiveness, our results underscore the potential importance of accurate, evidence-based prognostic tools in guiding clinical decisions. The objective of this study was to assess and compare the prognostic accuracy of the SOFA score, REDS score, and D-dimer levels in predicting 28-day mortality, using comprehensive and statistically rigorous methodologies. These findings contribute to the expanding literature on multidimensional and machine learning-assisted approaches in critical care and support further external validation in larger, diverse cohorts.

METHODS

This retrospective observational study was conducted at the Institute for Pulmonary Diseases of Vojvodina, within the Clinic for Intensive Care Medicine and Pulmonary Vascular Diseases, Department for Intensive Care and Intoxications Level 3. The study population included 163 critically ill patients with confirmed COVID-19 and sepsis who were admitted to the ICU between November 2020 and May 2022.

Eligibility criteria included adult patients (≥ 18 years) with COVID-19 confirmed via reverse transcription-polymerase chain reaction or rapid antigen testing using nasopharyngeal swabs. All patients met Sepsis-3 criteria, requiring documented or suspected infection and an acute increase of ≥ 2 points in the SOFA score [12]. To minimize confounding, we excluded individuals with advanced immunocompromised states (e.g., HIV/AIDS, transplant recipients, active cancer, autoimmune diseases), as well as pregnant or breastfeeding women.

Demographic, clinical, and laboratory data were collected from medical records within the first 24 hours of ICU admission. Variables necessary for calculating the SOFA and REDS scores were extracted from documentation and verified by two independent reviewers. Laboratory tests, including D-dimer levels, were performed using the VIDAS® D-Dimer Exclusion II assay (BioMérieux, Marcy-l'Étoile, France), which is based on an enzyme-linked fluorescent assay technique and analyzed on the VIDAS 3 platform.

The primary outcome was 28-day all-cause mortality. Statistical analyses were performed using SPSS Version 26.0 (IBM Corp., Armonk, NY, USA), MedCalc Statistical Software v20.2 (MedCalc Software Ltd., Ostend, Belgium). A two-tailed p-value < 0.05 was considered statistically significant.

Statistical analysis

Continuous variables were summarized as medians with interquartile ranges, while categorical variables were presented as frequencies and percentages. The distribution of continuous variables was assessed using the Shapiro–Wilk test. Between-group comparisons were conducted using the Mann–Whitney U test for non-normally distributed data and the χ^2 or Fisher's exact test for categorical variables, depending on expected cell frequencies. To evaluate the discriminatory power of the SOFA score, REDS score, and D-dimer levels for predicting 28-day mortality, ROC curve analyses were performed. The Youden index was applied to determine optimal cut-off values for each marker. Diagnostic performance was assessed through sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), each with corresponding 95% confidence intervals. Multivariate logistic regression analysis was conducted to identify independent predictors of 28-day mortality, with internal validation performed through bootstrapping (1000 iterations) to improve model robustness and minimize overfitting. Model calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test by comparing observed and expected mortality across deciles of predicted risk. Additionally, a CHAID decision tree analysis was employed to explore variable interactions and stratify mortality risk groups based on REDS, SOFA, and D-dimer, allowing identification of key thresholds and clinically relevant decision nodes.

Ethics: The study protocol received ethical approval from the Ethics Committee of the Institute for Pulmonary Diseases of Vojvodina (No. 9-II/3, February 24, 2022) and the Faculty of Medicine, University of Novi Sad (No. 01-39/190/1, May 13, 2022).

RESULTS

In this study involving 163 critically ill COVID-19 patients with sepsis, the prognostic performance of three early clinical indicators – SOFA score, REDS score, and D-dimer levels – was evaluated within the first 24 hours of ICU admission for their ability to predict 28-day mortality. In this cohort, the prevalence of comorbidities varied, with hypertension (55.8%) and diabetes mellitus (27%) being the most common (Figure 1). Although patients with malignancy exhibited the highest mortality rate (90.9%), this did not reach statistical significance (p = 0.062), likely due

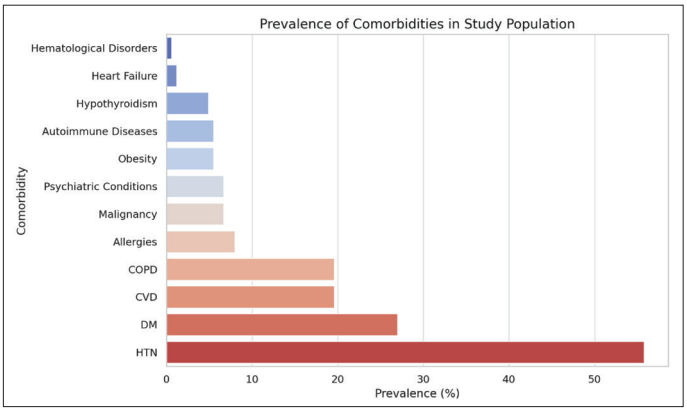


Figure 1. Prevalence of comorbidities in the patient cohort, with hypertension (HTN) (55.8%) and diabetes mellitus (DM) (27%) as the most frequent conditions; cardiovascular disease (CVD) and chronic obstructive pulmonary disease (COPD) rank third and fourth in prevalence, respectively; this distribution highlights the common occurrence of multiple underlying diseases that may influence patient prognosis and clinical management

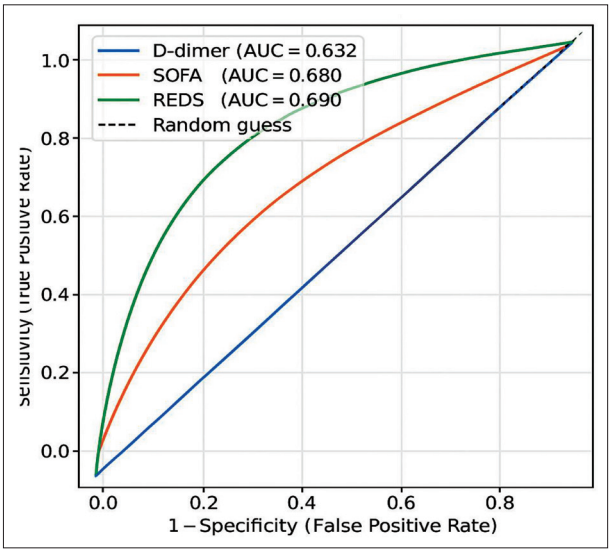


Figure 2. ROC curves for D-dimer, sequential organ failure assessment (SOFA), and risk-stratification of emergency department sepsis (REDS) scores showing their predictive accuracy for 28-day mortality; AUC values indicate the discriminative performance of each tool

to the small subgroup size. Other comorbidities such as chronic obstructive pulmonary disease, cardiovascular disease, and diabetes showed elevated mortality proportions but without statistically significant differences between survivors and deceased. The absence of significant associations may reflect limited statistical power or heterogeneous effects of individual comorbidities on 28-day outcomes. Overall, these findings suggest that while comorbid conditions are prevalent in critically ill patients, their isolated impact on short-term mortality requires further investigation in larger cohorts to clarify their prognostic value.

ROC curve analysis demonstrated moderate discriminative ability across all three parameters (Figure 2). The REDS score yielded the highest Youden index (0.293; 95% CI: 0.148–0.403), followed by the SOFA score (0.288; 95% CI: 0.162–0.420) and D-dimer (0.255; 95% CI: 0.132–0.377). The optimal cut-off values identified were REDS > 2, SOFA > 4, and D-dimer > 1425 μg/L. Notably, the confidence interval for D-dimer's cut-off value was wide (1028–8253 μg/L), indicating potential variability in its discriminatory capacity.

D-dimer (AUC = 0.632) shows modest predictive value, slightly better than chance. SOFA (AUC = 0.680) performs better with moderate accuracy. REDS (AUC = 0.690) is the best among the three, though only marginally superior to SOFA (Table 1). All models have AUCs between 0.6 and 0.7, indicating weak to moderate predictive power. Based on the ROC analysis for this sample, although none of the scores are strong predictors on their own, both REDS and SOFA demonstrated statistically significant differences between survivors and non-survivors ($p < 0.001$), highlighting their value as useful prognostic tools within a comprehensive clinical assessment.

Table 1. AUC ROC curve analysis for D-dimer, sequential organ failure assessment (SOFA) and risk-stratification of emergency department sepsis (REDS) scores for predicting 28-day mortality

Parameters	AUC ROC	SE	95% CI for AUC ROC	p
D-dimer	0.632	0.047	0.551–0.707	**0.005**
SOFA	0.680	0.042	0.603–0.751	**< 0.001**
REDS	0.690	0.043	0.613–0.760	**< 0.001**

SE – standard error; CI – confidence interval; p – probability that AUC differs from 0.5 (no discrimination); all AUC values 0.6–0.7 indicate moderate discrimination; p-values indicate significance of AUC versus 0.5 (no discrimination)

In terms of diagnostic accuracy, REDS > 2 demonstrated the highest sensitivity (80.2%) and NPV (71.3%), while SOFA > 4 achieved the highest specificity (56.1%) and PPV (75.5%). D-dimer > 1425 μg/L showed a sensitivity of 76.5%, specificity of 49.1%, PPV of 73.6%, and NPV of 52.9% (Table 2). Despite the observed differences in point estimates, the overlapping confidence intervals for

Table 2. Optimal cut-off, sensitivity, specificity, PPV and NPV for D-dimer, SOFA and REDS

Variable	Youden index	95% CI Youden index	Optimal cut-off value	95% CI for cut-off value	SENS (%)	SPEC (%)	PPV	NPV
D-dimer	0.25	0.13–0.37	> 1425	> 1028 – > 8253	76.47	49.09	73.6	52.9
SOFA	0.28	0.16–0.42	> 4	> 3 – < 6	72.64	56.14	75.5	52.5
REDS	0.29	0.14–0.40	> 2	> 1 – < 4	80.19	49.12	61.2	71.3

CI – confidence interval; SENS – sensitivity; SPEC – specificity; PPV – positive predictive value; NPV – negative predictive value; SOFA – sequential organ failure assessment; REDS – risk-stratification of emergency department sepsis

Table 3. Individual REDS parameters: comparison between survivors and non-survivors (n = 163)

REDS Component	Survivors n = 56	Non-survivors n = 107	χ^2 (df)	p
Age ≥ 65 years	24 (42.9%)	61 (57.0%)	2.95 (1)	0.086
GCS < 15	16 (28.6%)	55 (51.4%)	7.79 (1)	**0.005**
SBP < 100 mmHg	13 (23.2%)	28 (26.2%)	0.17 (1)	0.680
RR ≥ 22/min	30 (53.6%)	70 (65.4%)	2.18 (1)	0.140
Lactate (categorical: ≤ 2 / 2.1–3.9 / ≥ 4 mmol/L)	36 (64.3%) / 15 (26.8%) / 5 (8.9%)	42 (39.3%) / 46 (43.0%) / 19 (17.8%)	9.34 (2)	**0.009**
Albumin < 27 g/L	19 (34.5%)	41 (38.7%)	0.27 (1)	0.607
INR ≥ 1.3	17 (30.9%)	37 (34.9%)	0.26 (1)	0.610
Refractory hypotension + lactate: – RH absent – RH + lactate < 2 – RH + lactate ≥ 2.1	48 (85.7%) 5 (8.9%) 3 (5.4%)	72 (67.3%) 9 (8.4%) 26 (24.3%)	9.12 (2)	**0.010**

REDS – risk-stratification of emergency department sepsis; GCS – Glasgow coma scale; SBP – systolic blood pressure; RR – respiratory rate; INR – international normalized ratio; RH – refractory hypotension; lactate values are categorized as ≤ 2.0, 2.1–3.9, and ≥ 4.0 mmol/L; albumin < 27 g/L and INR ≥ 1.3 indicate hypoalbuminemia and coagulopathy, respectively; refractory hypotension in combination with elevated lactate (≥ 2.1 mmol/L) reflects the highest circulatory risk subgroup; χ^2 values with degrees of freedom (df) were used to assess the association between each REDS component and 28-day mortality

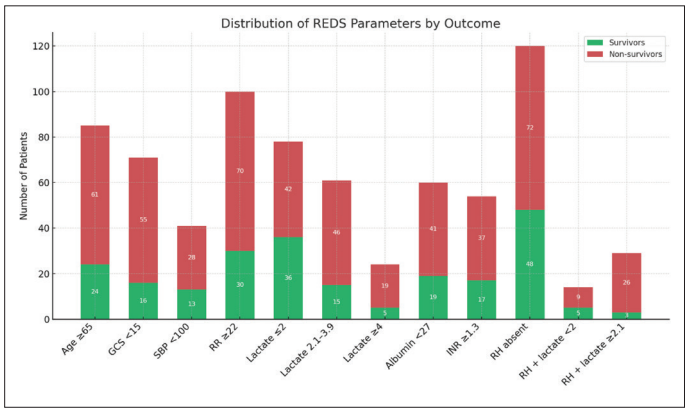


Figure 3. Distribution of risk-stratification of emergency department sepsis (REDS) parameters by outcome; lactate values are expressed in mmol/L, and albumin in g/L; GCS – Glasgow coma scale; SBP – systolic blood pressure; RR – respiratory rate; INR – international normalized ratio; RH – refractory hypotension

the Youden index suggest that none of the three markers was statistically superior in isolation.

In our research, three REDS parameters showed statistically significant associations with mortality outcomes (Table 3). Patients with Glasgow coma scale < 15 had over 75% mortality, indicating a strong correlation between severe neurological impairment and death. Similarly, approximately 80% of patients with lactate levels ≥ 4 mmol/L did not survive, underscoring the prognostic importance of elevated lactate. Furthermore, more than 85% of patients exhibiting refractory hypotension combined with lactate ≥ 2.1 mmol/L died, highlighting this combination as a potent clinical marker of high mortality risk. Among the eight REDS components, these variables demonstrated the most pronounced and significant differences between survivors and non-survivors, while other parameters such as systolic blood pressure < 100 mmHg and albumin < 27 g/L showed no significant discrimination (Figure 3). Despite variability in individual component performance, the composite REDS score exhibited superior discriminatory ability (AUROC = 0.69, $p < 0.001$) by integrating all variables, supporting its role as a comprehensive bedside risk stratification tool. These findings suggest that while certain REDS components have stronger individual prognostic value, the aggregate score remains the preferred measure for clinical application and may benefit from further optimization in future studies.

In the multivariate logistic regression model, both the REDS and SOFA scores were identified as independent predictors of mortality (Table 4). Each one-point increase in the REDS and SOFA scores was associated with a 22% increase in the odds of death (OR = 1.22, $p \leq 0.05$ for both). Male sex was also independently associated with increased mortality risk (OR = 2.78, p = 0.029). Other variables, including hypertension, diabetes, cardiovascular disease, chronic obstructive pulmonary disease, and allergies, were not statistically significant predictors in the adjusted model. Logistic regression analysis and combined ROC curves further supported the enhanced prognostic utility when these variables were used in tandem.

To assess the predictive value of clinical variables for mortality in critically ill COVID-19 patients with sepsis, a logistic regression analysis was performed using the bootstrap method with 1000 samples (Table 5). This approach allowed for a more robust estimation of the model parameters, reducing potential bias and improving the reliability of confidence intervals in small and potentially non-normally distributed datasets.

Table 4. Logistic regression summary table – multivariate model

Variable	B (Beta)	p	Exp(B) (OR)	Interpretation
SOFA score	0.204	0.024	1.226	Each one-point increase in SOFA raises death risk by 22.6%
D-dimer	0.000	0.050	1.000	Marginally significant; very weak or negligible effect
REDS score	0.203	0.050	1.225	Each one-point increase in REDS raises death risk by 22.5%
Sex (Male)	1.018	0.012	2.766	Males have 2.77 times higher risk of death compared to females
HTN	0.391	0.332	1.479	Not significant when adjusted for other variables
DM	0.147	0.755	1.159	Not significant
CVD	0.073	0.883	1.075	Not significant
COPD	0.724	0.145	2.062	Not statistically significant
Allergies	1.042	0.162	2.834	Not statistically significant

Logistic regression analysis of predictors of mortality in critically ill COVID-19 patients; B – regression coefficient; Exp(B) – odds ratio (OR); p-values ≤ 0.05 are considered statistically significant; in the multivariate logistic regression model, each 1-point increase in sequential organ failure assessment (SOFA) or risk-stratification of emergency department sepsis (REDS) scores was associated with an approximately 22% increase in the odds of 28-day mortality (OR = 1.226 for SOFA and OR = 1.225 for REDS; p ≤ 0.05 for both); male sex was also identified as an independent predictor of mortality, with males having nearly 2.8 times higher risk compared to females; other comorbidities [hypertension (HTN), diabetes mellitus (DM), cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD), allergies] did not show statistically significant associations with mortality in the multivariate model

Table 5. Multivariate logistic regression with bootstrap (1000 samples)

Variable	B (Coefficient)	Std. Error	p (Sig. 2-tailed)	95% CI – Lower	95% CI – Upper
SOFA score	0.188	0.077	0.005	0.044	0.355
D-dimer	0.000	0.000	0.143	0.000	0.000
REDS score	0.195	0.107	0.049	0.014	0.448
Constant	-1.421	0.525	0.003	-2.527	-0.447

(Outcome: in-hospital mortality) – multivariate logistic regression with bootstrap resampling (1000 samples) identified sequential organ failure assessment (SOFA) and risk-stratification of emergency department sepsis (REDS) scores as independent predictors of mortality in critically ill COVID-19 patients with sepsis; the 95% confidence intervals for SOFA and REDS excluded zero, supporting their prognostic relevance

Table 6. Observed vs. expected 28-day mortality by deciles of predicted risk

Decile of Predicted Probability	Observed Deaths (n)	Total Patients (n)	Observed Mortality (%)	Expected Deaths (n)	Expected Mortality (%)
1 (lowest risk)	5	16	31.3	10.5	65.6
2	5	16	31.3	10.5	65.6
3	10	17	58.8	11.2	65.9
4	10	16	62.5	10.5	65.6
5	9	16	56.3	10.5	65.6
6	15	17	88.2	11.2	65.9
7	11	16	68.8	10.5	65.6
8	15	17	88.2	11.2	65.9
9	14	16	87.5	10.5	65.6
10 (highest risk)	13	16	81.3	10.5	65.6
Total	107	163	65.6	107	65.6

The table displays the observed and expected mortality within each decile of predicted death probability; the "observed mortality (%)" column shows the actual mortality rate in each decile, while the "expected mortality (%)" reflects the model's predicted mortality; consistency between observed and expected mortality across deciles indicates good calibration of the predictive model

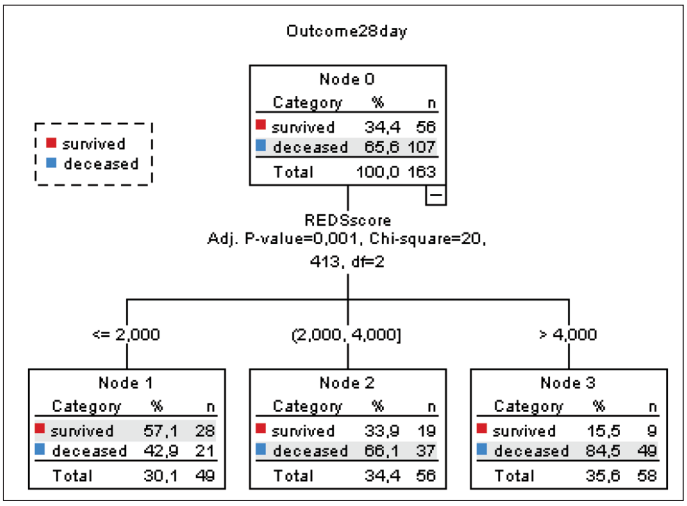


Figure 4. Decision tree model stratifying 28-day mortality outcomes using risk-stratification of emergency department sepsis (REDS) score

The use of bootstrap logistic regression represents a novel methodological aspect of this study, ensuring more stable estimates of the regression coefficients and confidence intervals compared to traditional methods. This technique enhances statistical robustness, particularly in clinical datasets with skewed distributions and heterogeneous patient populations. Our findings confirm the independent prognostic significance of both the SOFA and REDS scores, while D-dimer did not reach statistical significance in the multivariate model.

The calibration of the predictive model was evaluated across deciles of predicted mortality risk using the Hosmer–Lemeshow goodness-of-fit test, which yielded $p > 0.05$ for the overall model ($\chi^2 = 11.285$; $df = 8$; $p = 0.186$). This indicates that there was no statistically significant difference between the observed and expected mortality rates across all risk deciles (Table 6). The observed and expected mortality frequencies, as presented in Table 6, demonstrate satisfactory model calibration and internal validity, supporting its ability to stratify patients reliably across the full spectrum of disease severity. Such adequate calibration strengthens the model's clinical applicability by ensuring stable and trustworthy risk estimates that can aid early decision-making, optimize resource allocation, and guide individualized management strategies in critically ill patients.

The CHAID decision tree analysis was employed to identify the most relevant predictors of 28-day mortality in critically ill patients. Among the evaluated variables – SOFA score, REDS score, and D-dimer – only the REDS score remained in the final model, highlighting its important predictive value within this cohort (Figure 4). The resulting tree had a simple structure with one major split (depth = 1), dividing patients into three terminal nodes based on REDS score thresholds. Mortality rates increased progressively across these groups, from 42.9% in patients with REDS ≤ 2, to 66.1% in those with scores between 2.1–4.0, and reaching 84.5% in those with scores > 4.0. This stratification demonstrates the REDS score's strong discriminatory ability and practical clinical utility for early risk assessment. The simplicity of the model enhances its applicability in real-time decision-making; however, the absence of internal or external validation limits the ability to generalize these findings beyond the current sample and warrants further evaluation in broader patient populations.

DISCUSSION

This study provides important insights into the prognostic performance of early clinical indicators – SOFA score, REDS score, and D-dimer – in critically ill COVID-19 patients with sepsis. Consistent with prior research, our findings show that while none of these markers alone achieves ideal accuracy, the REDS score demonstrates the highest sensitivity and NPV, underscoring its value as an effective initial triage tool to identify low-risk patients. This aligns with the intended role of rapid assessment tools for early deterioration, highlighting their broader clinical applicability in patients with sepsis and the potential to improve timely interventions and resource allocation [7, 9, 11].

Conversely, the SOFA score exhibited superior specificity and PPV, reinforcing its utility in confirming patients at higher mortality risk. These complementary characteristics suggest that SOFA and REDS scores serve distinct but synergistic roles in risk stratification [12–15]. The D-dimer, despite moderate sensitivity, showed wide confidence intervals around cut-off points, reflecting biological variability and highlighting the challenges of relying on coagulation markers alone for prognostication in this context. This observation resonates with emerging literature documenting inconsistent predictive utility of D-dimer across heterogeneous COVID-19 cohorts [16–19].

Importantly, the overlapping confidence intervals for the Youden indices emphasize that these markers are best utilized in combination rather than isolation, supporting a multimodal prognostic strategy. Clinically, a REDS score > 2 may serve as an early alert prompting closer monitoring and resource allocation, whereas a SOFA score > 4 could guide escalation of care decisions. Incorporation of D-dimer into composite models may add incremental value but requires further validation [20, 21].

Our multivariate logistic regression analysis substantiates the independent prognostic significance of both REDS and SOFA scores, with each point increase correlating with a 22% rise in mortality odds, even after adjustment for key comorbidities. This reinforces the robustness of these scores as bedside tools reflecting organ dysfunction severity rather than mere comorbidity burden. Interestingly, common comorbidities including hypertension and diabetes were not independently predictive, suggesting their effects may be mediated through clinical deterioration captured by these scoring systems [22]. Male sex emerged as an additional independent risk factor, consistent with documented sex disparities in COVID-19 outcomes [23].

The CHAID decision tree analysis further illustrates the clinical utility of the REDS score by stratifying patients into clear mortality risk groups based on simple thresholds. In the era of precision medicine, such machine-learning–based models offer scalable, interpretable tools to augment clinician judgment and tailor management strategies [24]. Our findings support integrating decision tree models with validated scores like REDS to enhance real-time ICU triage, ultimately improving individualized care and resource optimization.

Calibration analysis is an important step in evaluating predictive models across diverse clinical populations [25, 26]. For example, it has been applied in neurological patients, and in our study, the model showed good alignment between observed and predicted 28-day mortality across risk deciles in critically ill COVID-19 patients with sepsis, supporting its potential reliability for early clinical decision-making.

During the COVID-19 pandemic, careful prioritization of emergency and elective cases became a critical aspect of hospital management, with clear guidelines emphasizing that only urgent, non-deferrable cases should be treated immediately to reduce hospital crowding and protect both patients and healthcare staff [27]. The identification of effective laboratory biomarkers that could stratify patients at risk of developing severe forms of the disease is imperative to ensure they receive prompt medical treatment [28]. In this context, the implementation of relatively simple and rapidly applicable scoring systems such as REDS and SOFA can facilitate the early identification of high-risk patients, enabling clinicians to prioritize treatment and optimize outcomes. These tools, together with biomarker such as D-dimer may play an important role in guiding appropriate and timely therapy in patients with severe forms of COVID-19 and sepsis.

Limitations of this study include its retrospective design, single-center setting, and modest sample size, which may affect generalizability. The absence of external validation warrants cautious interpretation and underlines the need for prospective, multicenter studies to confirm and refine these predictive models.

CONCLUSION

This study demonstrates that among critically ill COVID-19 patients with sepsis, the REDS score is a robust and independent predictor of 28-day mortality, outperforming both SOFA score and D-dimer levels in risk stratification. The application of CHAID decision tree analysis further confirms the REDS score's practical utility in categorizing patients into distinct mortality risk groups, facilitating timely clinical decision-making. Bootstrap-validated logistic regression reinforces the reliability of these findings despite the retrospective design and sample size limitations. These results support the integration of the REDS score into ICU protocols to improve early identification of high-risk patients and optimize resource allocation.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to Professors Ljiljana Andrijević, Ilija Andrijević, Bojan Zarić, and Jovan Matijašević for their valuable support and contribution to the realization of this research.

The results presented in this manuscript are part of the doctoral dissertation: Bajić D. *Prognostic Significance of Sepsis Biomarkers on the Outcome of Critically Ill Patients with COVID-19 Infection* [dissertation]. Novi Sad (Serbia): University of Novi Sad, Faculty of Medicine; 2024.

Conflict of interest: None declared.

REFERENCES

1. Lavrentieva A, Kaimakamis E, Voutsas V, Bitzani M. An observational study on factors associated with ICU mortality in Covid-19 patients and critical review of the literature. Sci Rep. 2023;13(1):7804. [DOI: 10.1038/s41598-023-34613-x] [PMID: 37179397]
2. Llitjos JF, Carrol ED, Osuchowski MF, Bonneville M, Scicluna BP, Payen D, et al. Enhancing sepsis biomarker development: key considerations from public and private perspectives. Crit Care. 2024;28(1):1–15. [DOI: 10.1186/s13054-024-05032-9] [PMID: 39003476]
3. Van den Berg M, van Beuningen FE, ter Maaten JC, Bouma HR. Hospital-related costs of sepsis around the world: A systematic review exploring the economic burden of sepsis. J Crit Care. 2022;71:154096. [DOI: 10.1016/j.jcrc.2022.154096] [PMID: 35839604]
4. Duncan CF, Youngstein T, Kirrane MD, Lonsdale DO. Diagnostic Challenges in Sepsis. Curr Infect Dis Rep. 2021;23(12):22. [DOI: 10.1007/s11908-021-00765-y] [PMID: 34720754]
5. Heubner L, Hattenhauer S, Güldner A, Petrick PL, Rößler M, Schmitt J, et al. Characteristics and outcomes of sepsis

patients with and without COVID-19. J Infect Public Health. 2022;15(6):670–6. [DOI: 10.1016/j.jiph.2022.05.008] [PMID: 35617831]
6. Thodphetch M, Chenthanakij B, Wittayachamnankul B, Sruamsiri K, Tangsuwanaruk T. A comparison of scoring systems for predicting mortality and sepsis in the emergency department patients with a suspected infection. Clin Exp Emerg Med. 2021;8(4):289–95. [DOI: 10.15441/ceem.20.145] [PMID: 35000356]
7. Sivayoham N, Mara HO, Turner NT, Sysum K, Wicks G, Mason O. Validation of the REDS score in hospitalised patients who deteriorated and were admitted to the intensive care unit – a retrospective cohort study. BMJ Open Qual. 2025;14(1):e003054. [DOI: 10.1136/bmjoq-2024-003054] [PMID: 39762056]
8. Sivayoham N, Hussain AN, Shabbo L, Christie D. An observational cohort study of the performance of the REDS score compared to the SIRS criteria, NEWS2, CURB65, SOFA, MEDS and PIRO scores to risk-stratify emergency department suspected sepsis. Ann Med. 2021;53(1):1863–74. [DOI: 10.1080/07853890.2021.1992495] [PMID: 34686088]
9. Sheerin T, Dwivedi P, Hussain A, Sivayoham N. Performance of the CURB65, NEWS2, qSOFA, SOFA, REDS, ISARIC 4C, PRIEST and the Novel COVID-19 Severity Scores, Used to Risk-Stratify Emergency Department Patients with COVID-19, on Mortality – An Observational Cohort Study. COVID. 2023;3(4):555–66. [DOI: 10.3390/covid3040040]
10. Kumar MCH, Chabba SK, Udasimath S, Ravishankar G, Sushma MKM, Gadwal NV. Study of Inflammatory Markers - CRP, D-dimer, and Ferritin in COVID- 19 Positive patients - A Retrospective Study. European Journal of Cardiovascular Medicine (EJCM) 2025;5(15):699–703. [DOI: 10.5083/ejcm/25-05-128]
11. Rahmoun M Al, Sabaté-elabbadi A, Guillemot D, Brun-buisson C, Watier L. Impacts of the COVID-19 pandemic on sepsis incidence, etiology and hospitalization costs in France: a retrospective observational study. BMC Infec Dis. 2025;25(1):627. [DOI: 10.1186/s12879-025-11000-7] [PMID: 40301806]
12. Esmaeili Tarki F, Afaghi S, Rahimi FS, Kiani A, Varahram M, Abedini A. Serial SOFA-score trends in ICU-admitted COVID-19 patients as predictor of 28-day mortality: A prospective cohort study. Heal Sci Reports. 2023;6(5):1–8. [DOI: 10.1002/hsr2.1116] [PMID: 37152236]
13. Lee HJ, Ko BS, Ryoo SM, Han E, Suh GJ, Choi SH, et al. Modified cardiovascular SOFA score in sepsis: development and internal and external validation. BMC Med. 2022;20(1):476. [DOI: 10.1186/s12916-022-02694-6] [PMID: 36482459]
14. Sherak RAG, Sajjadi H, Khimani N, Tolchin B, Jubanyik K, Taylor RA, et al. SOFA score performs worse than age for predicting mortality in patients with COVID-19. PLoS One. 2024;19(05):1–12. [DOI: 10.1371/journal.pone.0301013] [PMID: 38758942]
15. Munim FA, Yusof AM, Cheah SK, Khazrul M, Abd N, Rahiza W, et al. SOFA Score Trends in Predicting Mortality in Critically Ill COVID-19 Patients. COVID. 2025;5(9):154. [DOI: 10.3390/covid5090154]
16. Dumache R, Muresan CO, Maria S, Laitin D, Ivanovic N, Chisalita A, et al. COVID-19 Organ Injury Pathology and D-Dimer Expression Patterns: A Retrospective Analysis. Diagnostics. 2025;15(15):1860. [DOI: 10.3390/diagnostics15151860] [PMID: 40804825]
17. Benfathallah B, Boutagayout A, Hassani AC, Ihazmade H. Dynamic Relationship Between High D-Dimer Levels and the In-Hospital Mortality Among COVID-19 Patients: A Moroccan Study. COVID. 2025;5(8):116. [DOI: 10.3390/covid5080116]
18. Akerman M, Joseph DA. Role of peak D-dimer in predicting mortality and venous thromboembolism in COVID-19 patients. Sci Prog. 2025;108(1):368504241247982. [DOI: 10.1177/00368504241247982] [PMID: 40012497]
19. Bozorgmehr R, Shams N, Akbarniakhan H, Alirezaei T. D-dimer Levels as a Prognostic Inpatient Mortalit y Indicator in COVID-19 Patients: Insights from a Cross-Sectional Study. Int Jour of Cardiovas Prac. 2025;10(2):e162064. [DOI: 10.5812/intjcardiovascpract-162064]
20. Reddy V, Reddy H, Gemnani R, Kumar S, Acharya S. Navigating the Complexity of Scoring Systems in Sepsis Management: A Comprehensive Review. Cureus. 2024;16(2):e54030. [DOI: 10.7759/cureus.54030] [PMID: 38481909]
21. Silingardi M, Zappulo F, Dormi A, Pizzini AM, Donadei C, Cappuccilli M, et al. Is COVID-19 Coagulopathy a Thrombotic Microangiopathy? A Prospective, Observational Study. Int J Mol Sci. 2025;26(11):5395. [DOI: 10.3390/ijms26115395] [PMID: 40508203]
22. König S, Vaskyte U, Boesing M, Lüthi-corridori G, Leuppi JD. The Role of Comorbidities in COVID-19 Severity. Viruses. 2025;17(7):957. [DOI: 10.3390/v17070957] [PMID: 40733574]
23. Kaim A, Shetrit SB, Saban M. Women Are More Infected and Seek Care Faster but Are Less Severely Ill: Gender Gaps in COVID-19 Morbidity and Mortality during Two Years of a Pandemic in Israel. Healthcare. 2022;10(12):2355. [DOI: 10.3390/healthcare10122355] [PMID: 36553879]
24. Mohammadi-Pirouz Z, Hajian-Tilaki K, Haddat-Zavareh MS, Amoozadeh A, Bahrami S. Development of decision tree classifcation algorithms in predicting mortality of COVID-19 patients. Int J Emerg Med. 2024;17(1):126. [DOI: 10.1186/s12245-024-00681-7] [PMID: 39333862]
25. Poopipatpab S, Weerayutwattana R, Nuchpramool P, Phairatwet P. Evaluation of physiological severity scores for predicting COVID-19 disease progression: a retrospective study. BMC Infect Dis. 2025;25(1):758. [DOI: 10.1186/s12879-025-11127-7] [PMID: 40419986]
26. Lee YS, Han S, Lee YE, Cho J, Choi YK, Yoon SY, et al. Development and validation of an interpretable model for predicting sepsis mortality across care settings. Sci Rep. 2024;14(1):13637. [DOI: 10.1038/s41598-024-64463-0] [PMID: 38871785]
27. Ozturan B, Okay E, Yildiz Y, Iyetin Y, Demiroglu M, Ozkan K. Management of resources for orthopedic oncology and trauma patients during the COVID-19 pandemic – a retrospective cohort study. Srp Arh Celok Lek. 2022;150(3–4):138–42. [DOI: 10.2298/SARH210318027O]
28. Radojičić B, Dolić M, Taušan Đ, Radojičić M, Mišović M. Comparison of baricitinib and tocilizumab in clinical outcome among hospitalized patients with severe form of COVID-19 – our experiences. Srp Arh Celok Lek. 2025 (Online First) [DOI: 10.2298/SARH250108083R]

Прогностички значај *REDS*, *SOFA* и Д-димера код критично оболелих од ковида 19 са сепсом

Дејана Бајић[1], Милица Плазачић[2,3], Андреа Михајловић[4]

[1]Универзитет у Новом Саду, Медицински факултет, Катедра за биохемију, Нови Сад, Србија;
[2]Универзитет у Новом Саду, Медицински факултет, Катедра за педијатрију, Нови Сад, Србија;
[3]Институт за здравствену заштиту деце и омладине Војводине, Нови Сад, Србија;
[4]Универзитет у Новом Саду, Медицински факултет, Катедра за физиологију, Нови Сад, Србија

САЖЕТАК

Увод/Циљ Рано препознавање болесника са високим ризиком од смртног исхода код сепсе настале услед ковида 19 од суштинског је значаја за правовремену интервенцију и оптималну расподелу ресурса у јединици интензивног лечења. Циљ рада био је да се процени и упореди прогностичка вредност скорова *REDS* (Скор за стратификацију ризика сепсе на одељењу хитне помоћи) и *SOFA* (Скор процене секвенцијалног попуштања органа), заједно са нивоом Д-димера, у предикцији 28-дневног морталитета код тешко оболелих болесника.

Методе Ретроспективна анализа обухватила је 163 одрасла болесника са потврђеним ковидом 19 и сепсом (критеријуми Сепса-3), лечена у јединици интензивног лечења терцијарног центра у периоду од новембра 2020. до маја 2022. *REDS*, *SOFA* и вредности Д-димера процењене су у року од 24 сата од пријема. Прогностичка вредност анализирана је помоћу *ROC* анализе, логистичке регресије аутоматским детектором интеракција (Хи-квадрат) (*CHAID*) моделовања стабла одлуке.

Резултати Укупна 28-дневна смртност износила је 65,6%. *REDS* (*AUC* = 0,690) и *SOFA* (*AUC* = 0,680) показали су умерену предиктивну способност, док је Д-димер имао нижу тачност (*AUC* = 0,632). *REDS* > 2 имао је највећу сензитивност (80,2%), док је *SOFA* > 4 показао највећу специфичност (56,1%). *REDS* и *SOFA* идентификовани су као независни предиктори смртности. *CHAID* анализа издвојила је *REDS* као најјачи дискриминатор, који је стратификовао болеснике у три групе ризика са стопама смртности од 42,9%, 66,1% и 84,5%.

Закључак Скорови *REDS* и *SOFA* имају значајну прогностичку вредност код болесника са ковидом 19 повезаним сепсом. *REDS* је показао благу предност и може послужити као једноставан и ефикасан алат за рану стратификацију ризика у клиничкој пракси и будућим вирусним пандемијама.

Кључне речи: ковид 19; сепса; скор *REDS*; скор *SOFA*; прогноза; морталитет

DOI: https://doi.org/10.2298/SARH250108083R
UDC: 616.24-085.37; 616.98:578.834

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparison of baricitinib and tocilizumab in clinical outcome among hospitalized patients with severe form of COVID-19 – our experiences

Bojan Radojičić[1,2], Mirko Dolić[1], Đorđe Taušan[1,3], Marija Radojičić[4,5], Miroslav Mišović[3], Dejan Kostić[3]

[1]Karaburma Military COVID Hospital, Belgrade, Serbia;
[2]Visan – College of Health and Sanitary Vocational Studies, Belgrade, Serbia;
[3]Military Medical Academy, Belgrade, Serbia;
[4]Dr. Simo Milošević Health Center, Belgrade, Serbia;
[5]Dr. Simo Milošević COVID Ambulance, Belgrade, Serbia

SUMMARY

Introduction/Objective Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection manifests with an unpredictable clinical course that could rapidly progress, leading to serious and often fatal complications. The identification of effective laboratory biomarkers that could stratify patients at risk of developing severe forms of the disease is imperative to ensure they receive prompt medical treatment. The aim of our study was to compare baricitinib and tocilizumab regarding clinical outcomes in hospitalized patients with severe COVID-19.

Methods A retrospective study included analysis of data from 82 patients of both sexes who were treated with biological immunosuppressive therapy at the Military COVID Hospital, Karaburma. All patients who met the criteria for use according to the guidelines of the national protocol for the treatment of COVID-19, version XIII, had a consultative decision made for the application of biological immunosuppressive therapy. Half were treated with tocilizumab, and the other half with baricitinib.

Results The results of our study show that the mortality rate is lower in the group treated with tocilizumab compared with the group treated with baricitinib: 17.1% *vs.* 26.8%, but without statistical significance (p = 0.286). Additionally, the use of tocilizumab reduces the need for mechanical ventilation compared with baricitinib: 53.6% *vs.* 68.3%, without statistical significance (p = 0.454).

Conclusion The results obtained in our study indicate that both drugs are equally clinically effective.

Keywords: COVID-19; clinical outcome; cytokine storm; baricitinib; tocilizumab

INTRODUCTION

The pandemic caused by coronavirus (COVID-19) was the greatest scientific, medical, and social challenge since the Spanish flu pandemic of 1918. Severe acute respiratory syndrome caused by coronavirus 2 (SARS-CoV-2) manifests with an unpredictable clinical course that could rapidly progress, leading to serious and often fatal complications. The identification of effective laboratory biomarkers that could stratify patients at risk of developing severe forms of the disease is imperative to ensure they receive prompt medical treatment. One such biomarker is interleukin (IL)-6. It belongs to a group of molecules known as cytokines. These are part of the "inflammatory cascade," which involves coordinated, sequential activation of immune response pathways and are responsible for the onset of a "cytokine storm" – a hyperinflammatory response to infection that significantly impacts mortality in COVID-19 patients [1].

Ponti et al. [2] emphasized in a 2020 study the role of systemic vasculitis and cytokine-mediated coagulation disorders as key contributors to multiple organ failure in patients with severe forms of COVID-19. The following biomarkers have been identified as correlating with an increased risk of developing disseminated intravascular coagulation and acute respiratory distress syndrome. Hematological markers: lymphocyte count, neutrophil count, and their ratio – the neutrophil-to-lymphocyte ratio (NLR); inflammatory markers: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin; immunological marker: IL-6, and biochemical markers: D-dimer, troponin, creatine kinase, and aspartate aminotransferase (AST). Elevated concentrations of IL-6 have been shown to be a significant factor in damaging the host immune response in COVID-19 by stimulating a strong pro-inflammatory response, leading to a cytokine storm – a hyperinflammatory response to infection that has a significant impact on mortality in COVID-19 patients. Therefore, immunomodulatory therapies targeting IL-6 receptor antagonism have been explored as a countermeasure to host immune dysregulation and to support the beneficial effects of corticosteroids.

Tocilizumab is a recombinant humanized monoclonal antibody. It acts as an IL-6 receptor antagonist. Various observational studies have reported the beneficial effects of tocilizumab. Furthermore, subsequent randomized controlled trials have shown significant positive

Received • Примљено: January 8, 2025
Revised • Ревизија: September 16, 2025
Accepted • Прихваћено: October 22, 2025
Online first: October 30, 2025

Correspondence to:
Bojan RADOJIČIĆ
Tošin Bunar 7a
11080 Beograd, Serbia
dr.bojan.r@gmail.com

Table 1. General characteristics of patients treated with biological immunosuppressive therapy

General characteristics of patients	Treatment outcome						Test
	[All]		Survivors		Fatal outcome		p
	N = 82		N = 64		N = 18		
Sex							0.577[a]
Male	59	(72%)	47	(73.4%)	12	(66.7%)	
Female	23	(28%)	17	(26.6%)	6	(33.3%)	
Age (years) Median (min–max)	60	(24–81)	59	(24–81)	64	(31–78)	0.034[b]
Medication							0.284[a]
Baricitinib	41	(50%)	30	(46.9%)	11	(26.83%)	
Tocilizumab	41	(50%)	34	(53.1%)	7	(17.07%)	
Vaccination							0.309[a]
Yes	21	(25.6%)	18	(85.7%)	3	(14.3%)	
No	61	(74.4%)	46	(74.4%)	15	(24.6%)	
							0.388[a]
Sinopharm	18	(85.7%)	16	(88.9%)	2	(66.7%)	
Pfizer	2	(9.5%)	1	(5.6%)	1	(33.3%)	
Sputnik	1	(4.8%)	1	(5.6%)	0	(0%)	
							0.182[a]
ICU stay, days median (min–max)	10	(3–44)	9	(3–44)	11	(4–24)	0.642[b]
Mechanical ventilation							
Invasive mechanical ventilation	18	(37.5%)	0	(0%)	18	(100%)	< 0.001[a]
Full-face mask	30	(62.5%)	30	(100%)	0	(0%)	

[a]Likelihood ratio test;
[b]Mann–Whitney test

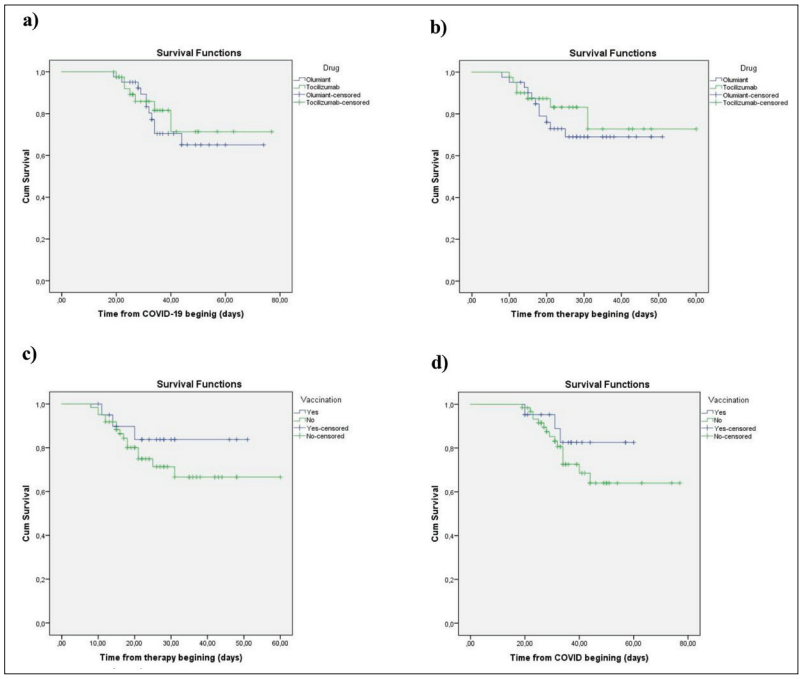


Figure 1. Survival curves;
a) treatment (from the onset of illness to outcome) of patients treated with baricitinib and tocilizumab [log rank (Mantel–Cox) = 0.240, df = 1, p = 0.624]; b) treatment (from the start of biological therapy to outcome) of patients treated with baricitinib and tocilizumab [log rank (Mantel–Cox) = 0.433, df = 1, p = 0.510]; c) treatment (from the onset of illness to outcome) in vaccinated and unvaccinated patients [log rank (Mantel–Cox] = 0.952, df = 1, p = 0.329); d) treatment (from the start of biological therapy to outcome) in vaccinated and unvaccinated patients [log rank (Mantel–Cox] = 0.889, df = 1, p = 0.3

results regarding the efficacy and safety of tocilizumab, focusing on outcomes such as mortality, risk of admission to intensive care units, and the need for mechanical ventilation. A meta-analysis conducted by Wei et al. [3] indicates that the introduction of tocilizumab in the treatment of patients with severe COVID-19 is associated with a lower risk of fatal outcomes and the need for mechanical ventilation.

In January 2022, the World Health Organization (WHO) [4] recommended two new drugs for COVID-19, providing new options for treating more severe forms of the disease. One of them was baricitinib, part of a class of drugs known as Janus kinase inhibitors (JAK1 and 2), which suppress excessive immune system stimulation (an oral medication used to treat rheumatoid arthritis by blocking IL-6 signaling), and it was strongly recommended for patients with severe or critical forms of COVID-19. WHO recommends that it be administered together with corticosteroids.

The main problem with biological therapy is the cost and availability of the drugs. WHO is in negotiations with manufacturers to ensure global supply capacity and equitable and sustainable access to newly recommended therapeutic agents. The Access to COVID-19 Tools Accelerator therapeutic pillar is engaged with pharmaceutical companies to seek comprehensive access plans for low- and middle-income countries so that these treatments can be quickly implemented everywhere, not just in wealthy nations.

METHODS

This research was conducted at the Karaburma Military COVID Hospital in the period September 1 – December 31, 2021, and it had a retrospective character. The aim of our study was to compare the efficacy of baricitinib and tocilizumab on mortality among hospitalized patients with a severe form of COVID-19. We also investigated whether sex, cardiovascular comorbidities, diabetes, elevation of lactate dehydrogenase (LDH), D-dimer, transaminases, NLR index, vaccination, and age affect mortality.

Table 2. Clinical characteristics of patients treated with biological immunosuppressive therapy

Clinical characteristics	Treatment outcome			Test
	[ALL]	Survivors	Fatal outcome	p
	N = 82	N = 64	N = 18	
Time from onset of illness to hospitalization (days)	7 (1–15)	7 (1–15)	7 (1–15)	0.282[b]
Time from onset of illness to start of treatment (days)	11 (5–26)	11 (5–26)	13 (9–26)	0.034[b]
Duration of treatment	22 (8–60)	23.5 (10–60)	15.5 (8–31)	< 0.001[b]
Time from start to end of treatment (days)	34 (19–77)	35.5 (19–77)	30 (19–44)	0.009
IL-6 (values at the beginning of th)	98.4 (41.1–366)	99.7 (41.1–333)	95.4 (42.1–366)	0.960[b]
CRP	125.5 (29–243)	122 (29–243)	129.5 (44–194)	0.758[b]
D-dimer	2 (0.41–34.55)	1.9 (0.41–34.55)	13.2 (1.28–34.47)	< 0.001[b]
ESR	60 (26–109)	64 (26–109)	56 (30–102)	0.023[b]
AST	57 (5–663)	53 (5–334)	70 (19–663)	0.330[b]
ALT	84 (21–817)	89 (21–451)	76.5 (24–817)	0.519[b]
LDH	466 (193–2743)	435.5 (193–1042)	598.5 (354–2743)	0.001[b]
ALB	35 (21–46)	35.5 (31–46)	33.5 (21–44)	0.210[b]
WBC	9.3 (2.27–45.9)	9 (2.27–45.9)	11.5 (4.3–23.3)	0.010[b]
Lymphocytes	0.6 (0.2–40.9)	0.6 (0.2–0.9)	0.45 (0.2–1.2)	0.133[b]
Lymphocytes				0.048[a]
< 0.5	25 (30.5%)	16 (25.0%)	9 (50%)	
others	57 (69.5%)	48 (75.0%)	9 (50%)	
Clostridia				0.229[a]
Yes	17 (20.7%)	15 (88.3%)	2 (11.7%)	
No	65 (79.3%)	49 (75.4%)	16 (24.6%)	
Cardiovascular disease				0.373[a]
Yes	52 (63.4%)	39 (75.0%)	13 (25%)	
No	30 (36.6%)	25 (83.3%)	5 (16.7%)	
Diabetes mellitus				0.332[a]
Yes	16 (19.5%)	11 (68.25%)	5 (31.25%)	
No	66 (80.5%)	53 (82.8%)	13 (19.7%)	

CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; AST – aspartate aminotransferase; ALT – alanine aminotransferase; LDH – lactate dehydrogenase; ALB – albumin; WBC – white blood cell (count);
[a]Likelihood ratio test;
[b]Mann–Whitney test;
Note: numerical variables shown as median (min–max)

In our study we indicated the application of biological therapy based on the version XIII of the COVID-19 Treatment Protocol [5]. Tocilizumab was administered at a dose of 8 mg/kg i.v. per dose. Two doses were given (with a maximum of 800 mg per dose). The administration of baricitinib depended on the eGFR value. The duration of therapy was limited to a maximum of 14 days or until clinical improvement occurred, if it happened within two weeks. For eGFR ≥ 60 mL/min/1.73 m², baricitinib was administered at 4 mg once daily; for eGFR 30 to < 60 mL/min/1.73 m², 2 mg daily; for eGFR 15 to < 30 mL/min/1.73 m², 1 mg daily. Baricitinib was not recommended for eGFR < 15 mL/min/1.73 m², and no such patients were included in the study.

IBM SPSS Statistics, Version 24.0 (IBM Corp., Armonk, NY, USA) was used for statistical data processing. Numerical features are presented through means (median, arithmetic mean) and measures of variability (standard deviation, range of values). Attribute features are shown using frequencies and percentages. The likelihood ratio test and Mann–Whitney test were used to test the relationship between two categorical variables. The value of significance level $p \leq 0.05$ was considered statistically significant.

Ethics: The study was approved by the Ethics Committee of the Military Medical Academy, Belgrade (approval no. 48/2025) and complied with the revised ethical guidelines of the Declaration of Helsinki.

RESULTS

The study included 82 patients treated with biological immunosuppressive therapy, of which half, or 41 patients, were treated with tocilizumab (Actemra), and the other half with baricitinib (Olumiant, Eli Lilly and Company, Indianapolis, IN, USA). The age range of the patients was 24–81 years, with a median of Me = 60 years. The proportion of male patients was significantly higher, at 72%. A quarter of the patients were vaccinated (25.6%), with a fatal outcome recorded in 14.3% of vaccinated and 24.6% of unvaccinated patients.

In the intensive care unit, patients spent an average of Me = 10 days, with 37.5% being intubated. The fatal outcome occurred in older patients (Me = 64) compared to survivors, who were on average younger (Me = 59), but without statistical significance (p = 0.034). Mechanical ventilation

Table 3. Mortality rate and mechanical ventilation between two patient groups

Parameter	Drug			Test
	[All]	Baricitinib	Tocilizumab	p
	N = 82	N = 41	N = 41	
Treatment outcome				0.286[a]
Survivors	64 (78%)	30 (73.2%)	34 (82.9%)	
Fatal outcome	18 (22%)	11 (26.8%)	7 (17.1%)	
Mechanical ventilation				
Invasive mechanical ventilation	18 (37.5%)	11 (42.3%)	7 (31.8%)	0.454[a]
Full-face mask	30 (62.5%)	15 (57.7%)	15 (68.2%)	

[a]Likelihood ratio test

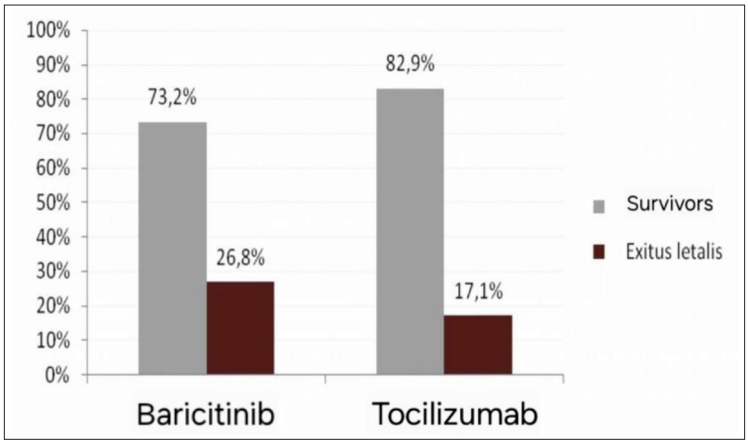


Figure 2. Mortality rate

was applied in 68.3% of patients who received baricitinib and in 53.6% of patients who received tocilizumab.

Patient hospitalization occurred on average on the seventh day, while treatment began on the 11th day. Treatment started earlier in surviving patients (Me = 11) compared to those with a fatal outcome (M = 13), p = 0.034. Survivors were treated on average for M = 23.5 days, while those who died were treated for an average of Me = 15.5 days, p < 0.001. The total time from the onset of illness to the final outcome (discharge or death) was Me = 35.5 days for the survivor group, and Me = 30 days for the group with a fatal outcome, p = 0.009.

The levels of IL-6, ESR, and CRP were similar in the compared groups. D-dimer levels were significantly higher in those who died (Me = 13.16) compared to those who survived (Me = 1.9), p < 0.001.

The values of liver enzymes – alanine aminotransferase – were similar between the groups, while AST was higher in the group with fatal outcomes (Me = 70 *vs.* Me = 53), although without statistical significance. LDH levels were significantly higher in patients with a fatal outcome (Me = 598.5), p = 0.001.

A reduced lymphocyte count, or lymphopenia, was present in all 82 patients. The NLR index in all deceased patients was > 10. Of the total number of deceased patients, 64.3% had lymphocyte values below 0.5×10^6, and 93% had values below 0.7×10^6.

Clostridia infection was present in 20.7% of patients receiving biological therapy, and the study results did not indicate that it had an impact on mortality.

The results of our study show that the mortality rate was lower in the group treated with tocilizumab compared to the group treated with baricitinib – 17.1% *vs.* 26.8%, but without statistical significance (p = 0.286). Additionally, the use of tocilizumab reduced the need for mechanical ventilation compared to baricitinib – 53.6% *vs.* 68.3%, without a statistically significant difference (p = 0.454).

Survival time (measured from the start of receiving biological therapy) for patients on baricitinib is M = 40.5, and for those on tocilizumab M = 49.3 days, but without a statistically significant difference. Total survival time measured from the start of biological therapy of all patients is M = 47.9 days. Survival time measured from the onset of the disease for vaccinated patients is M = 54.5, and for non-vaccinated patients M = 60.7 days, but without a statistically significant difference. Survival time (measured from the start of receiving biological therapy) for vaccinated patients is M = 45.2, and for unvaccinated M = 46.2 days, also without statistically significant difference. The log rank test shows that there are no statistically significant differences in treatment duration between vaccinated and unvaccinated patients, as well as between patients on tocilizumab and baricitinib. Furthermore, survival among vaccinated patients was somewhat higher compared to unvaccinated patients – 85.7% *vs.* 74.4%, but without statistical significance (p = 0.388).

DISCUSSION

In our study, the criteria for biological therapy were as follows: stages III and IV of the disease, with progression of lung findings and/or an increase in inflammatory markers, IL-6 levels ≥ 40 ng/l, and CRP > 50 mg/l, after the administration of adequate corticosteroid therapy without effect, respiratory rate ≥ 25 breaths/minute, SpO_2 (pulse oximetry) < 90%, and pO_2 < 8.66 kPa on ambient air (without oxygen support). Similar recommendations are given in the Spanish treatment protocol for COVID-19 [6] when biological therapy is included in the event of respiratory insufficiency and an increase in IL-6 levels > 15 ng/l.

In a retrospective cohort study conducted by Conroy et al. [7], it is concluded that there was no difference in mortality or adverse effects between tocilizumab and baricitinib. The results of our study show that the mortality rate was somewhat lower in the group treated with tocilizumab compared to the group treated with baricitinib – 17.1% *vs.* 26.8%, but without statistical significance (p = 0.286). The same conclusion was reached in the study by Cawcutt and Kalil [8]. In a retrospective cohort study of the National Covid Collaborative [9], which includes more than 10,000

patients treated in the USA, baricitinib was associated with lower hospital mortality and shorter hospital length of stay compared with tocilizumab. Marconi et al. [10] evaluated the use of baricitinib in the COV-BARRIER study in a randomized, placebo-controlled, double-blind trial. The study's randomization was carried out strictly through an interactive response system and stratified according to disease severity, age, region, and steroid use. The trial was conducted in 101 centers across 12 countries and included 1525 patients. Compared to the placebo, patients receiving baricitinib had a relative reduction of 38.2% and an absolute reduction of 5% in 28-day all-cause mortality. No other COVID-19 therapy has demonstrated such a significant reduction in mortality. For comparison, the other two immunomodulatory treatments associated with reduced mortality were dexamethasone – which showed a 17% relative reduction and a 2.8% absolute reduction in mortality – and tocilizumab – which showed a 15% relative reduction and a 4% absolute reduction in mortality. Further studies will provide clearer guidelines for optimal treatment for COVID-19 patients.

The conclusion of the study by Choi [11] is that mortality is up to two times higher in men compared to women, which is consistent with the results of our study – 66.7% *vs.* 33.3%. Additionally, mortality is up to five times higher in individuals with comorbidities compared to healthy individuals. In our study, cardiovascular comorbidities were recorded in 63.4% of patients receiving biological therapy, with a mortality rate of 25%, whereas in the population without cardiovascular disease, mortality was 16.7%. Diabetes was present in 19.5% of patients, with a mortality rate of 31.2% among diabetics, which is significantly higher than the 19.7% mortality rate in non-diabetic patients. Twelve patients had combined comorbidities, six in each group. Ten patients with diagnosed hypertension and diabetes (86% of them) required non-invasive ventilation or full-face mask, leading us to conclude that dual comorbidities significantly increase the risk of respiratory failure and the need for mechanical ventilation.

NLR has proven its prognostic value in infections. It is considered that the normal NLR range is 0.78–3.53 [12]. A meta-analysis by Ulloque-Badaracco et al. [13] in 2021 indicates that NLR can be a useful prognostic biomarker in patients with severe forms of COVID-19, and that high NLR values may indicate a poor prognosis in these patients. The NLR index in all deceased patients in our study was > 10.

A meta-analysis conducted by Tleyjeh et al. [14] concluded that the use of tocilizumab reduces the risk of mechanical ventilation in hospitalized patients with COVID-19, which is also a result obtained in our study. Tocilizumab reduced the need for mechanical ventilation compared to baricitinib – 53.6% *vs.* 68.3%, without a statistically significant difference (p = 0.454). Similar results were obtained in the study by Reid et al. [15].

CONCLUSION

The results obtained in our study indicate that both drugs are equally clinically effective, meaning that there is no statistically significant difference in the mortality rate (p = 0.286) between patients treated with tocilizumab and those treated with baricitinib.

Male sex, comorbidities, particularly dual comorbidities (diabetes mellitus and cardiovascular disease), elevated LDH and D-dimer values, NLR index > 10, and hypoalbuminemia are unfavorable prognostic factors, while vaccination status, elevated transaminases, and age do not impact mortality.

The obtained results should be confirmed in future studies to ensure optimal treatment for this group of severely ill patients.

Conflicts of interest: None declared.

REFERENCES

1. Hu B, Huang S, Yin L. The cytokine storm and COVID-19. J Med Virol. 2021;93(1):250–6. [DOI: 10.1002/jmv.26232] [PMID: 32592501]
2. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci. 2020;57(6):389–99. [DOI: 10.1080/10408363.2020.1770685] [PMID: 32503382]
3. Wei Q, Lin H, Wei RG, Chen N, He F, Zou DH, et al. Tocilizumab treatment for COVID-19 patients: a systematic review and meta-analysis. Infect Dis Poverty. 2021;10(1):71. [DOI: 10.1186/s40249-021-00857-w] [PMID: 34001244]
4. World Health Organization. WHO recommends two new drugs to treat COVID-19 [Internet]. 2022 [cited 2025]. Available from: https://www.who.int/news/item/14-01-2022-who-recommends-two-new-drugs-to-treat-covid-19
5. Ministry of Health of the Republic of Serbia. National protocol for the treatment of COVID-19, version XIII [Internet]. [cited 2025]. Available from: https://www.dzsvenac.rs/sluzbeno/images/dokumenta/covid/Terapijski-protokol-covid-19_122021_verzija13.pdf
6. Hernández García G, Barbero Allende JM, García Sánchez F, Sanz Moreno J. Treatment protocol for COVID-19 in patients with and without an indication for hospital admission. Medicine (Madr). 2022;13(55):3246–9. [DOI: 10.1016/j.med.2022.05.005] [PMID: 35582698]
7. Conroy GM, Bauer SR, Pallotta AM, Duggal A, Wang L, Sacha GL. Baricitinib versus tocilizumab in critically ill COVID-19 patients: a retrospective cohort study. Pharmacotherapy. 2024;44(1):28–38. [DOI: 10.1002/phar.2924] [PMID: 37593883]
8. Cawcutt KA, Kalil AC. Baricitinib or tocilizumab? Treatment of patients hospitalized with severe COVID-19. Crit Care Med. 2023;51(3):413–5. [DOI: 10.1097/CCM.0000000000005757] [PMID: 36809262]
9. Patanwala AE, Xiao X, Hills TE, Higgins AM, McArthur CJ, Alexander GC, et al. Comparative effectiveness of baricitinib versus tocilizumab in hospitalized patients with COVID-19: a retrospective cohort study of the National COVID Cohort Collaborative. Crit Care Med. 2025;53(1):e29–41. [DOI: 10.1097/CCM.0000000000006444] [PMID: 39365115]
10. Marconi VC, Ramanan AV, Bono S, Kartman C, Krishnan V, Liao R, et al. Efficacy and safety of baricitinib for the treatment of hospitalised adults with COVID-19 (COV-BARRIER): a randomised, double-blind, parallel-group, placebo-controlled phase 3 trial. Lancet Respir Med. 2021;9(12):1407–18. [DOI: 10.1016/S2213-2600(21)00331-3] [PMID: 34480861]
11. Choi WY. Mortality rate of patients with COVID-19 based on underlying health conditions. Disaster Med Public Health Prep. 2022;16(6):2480-5. [DOI: 10.1017/dmp.2021.139] [PMID: 33934734]

12. Forget P, Khalifa C, Defour JP, Latinne D, Van Pel MC, De Kock M. What is the normal value of the neutrophil-to-lymphocyte ratio? BMC Res Notes. 2017;10(1):12. [DOI: 10.1186/s13104-016-2335-5] [PMID: 28057051]
13. Ulloque-Badaracco JD, Salas-Tello WI, Al-Kassab-Córdova A, Alarcón-Braga EA, Benites-Zapata VA, Maguiña JL, et al. Prognostic value of neutrophil-to-lymphocyte ratio in COVID-19 patients: a systematic review and meta-analysis. Int J Clin Pract. 2021;75(11):e14596. [DOI: 10.1111/ijcp.14596] [PMID: 34228867]
14. Tleyjeh IM, Kashour Z, Damlaj M, Riaz M, Tleyjeh H, Altannir M, et al. Efficacy and safety of tocilizumab in COVID-19 patients: a living systematic review and meta-analysis. Clin Microbiol Infect. 2021;27(2):215–27. [DOI: 10.1016/j.cmi.2020.10.036] [PMID: 33161150]
15. Reid NK, Joyner KR, Lewis-Wolfson TD. Baricitinib versus tocilizumab for the treatment of moderate to severe COVID-19. Ann Pharmacother. 2023;57(7):769–75. [DOI: 10.1177/10600280221133376] [PMID: 36314277]

Поређење клиничког исхода барицитиниба и тоцилизумаба код хоспитализованих болесника са тешком формом ковида 19 – наша искуства

Бојан Радојичић[1,2], Мирко Долић[1], Ђорђе Таушан[1,3], Марија Радојичић[4,5], Мирослав Мишовић[3], Дејан Костић[3]

[1]Војна ковид болница „Карабурма“, Београд, Србија;
[2]Висока здравствено-санитарна школа струковних студија „Висан“, Београд, Србија;
[3]Војномедицинска академија, Београд, Србија;
[4]Дом здравља „Др Симо Милошевић“, Београд, Србија;
[5]Ковид амбуланта „Др Симо Милошевић“, Београд, Србија

САЖЕТАК

Увод/Циљ Тешки акутни респираторни синдром изазван коронавирусом (*SARS-CoV*-2) манифестовао се непредвидивим клиничким током болести, који се могао брзо развијати, изазивајући озбиљне и често смртоносне компликације. Идентификација ефикасних лабораторијских биомаркера који би могли да стратификују болеснике према ризику од појаве тешких облика болести императив је како би им се обезбедио брз медицински третман.

Циљ наше студије је упоређивање ефикасности барицитиниба и тоцилизумаба у клиничком исходу код хоспитализованих болесника са тешком формом ковида 19.

Методе Ретроспективном студијом обухваћена је анализа података 82 болесника оба пола који су лечени биолошком имуносупресивном терапијом у Војној ковид болници „Карабурма“. За све болеснике који су испуњавали критеријуме за њену примену према смерницама Националног протокола за лечење ковида 19 (верзија XIII) донета је конзилијарна одлука о примени биолошке имуносупресивне терапије. Половина болесника лечена је тоцилизумабом, а друга половина барицитинибом.

Резултати Резултати наше студије показују да је стопа морталитета нижа код групе болесника лечених тоцилизумабом у односу на групу лечену барицитинибом – 17,1% *vs.* 26,8%, али без статистичке значајности ($p = 0,286$). Такође, примена тоцилизумаба смањила је потребу за механичком вентилацијом у односу на барицитиниб – 53,6% *vs.* 68,3%, без статистичке значајности ($p = 0,454$).

Закључак Резултати наше студије указују да су оба лека подједнако клинички ефикасна.

Кључне речи: ковид 19; клинички исход; цитокинска олуја; барицитиниб; тоцилизумаб

DOI: https://doi.org/10.2298/SARH250723081D
UDC: 616.314.2-007-089

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Impact of orthognathic surgery on occlusal contacts and bite force distribution in patients with skeletal class III malocclusion by T-Scan™ – prospective clinical pilot study

Amar Đerlek[1], Neda Stefanović[1], Zorana Stamenković[1], Tina Pajević[1], Ivan Arsić[1], Biljana Miličić[2], Nenad Nedeljković[1]

[1]University of Belgrade, School of Dental Medicine, Department of Orthodontics and Dentofacial Orthopedics, Belgrade, Serbia;
[2]University of Belgrade, School of Dental Medicine, Department of Medical Statistics and Infor-matics, Belgrade, Serbia

SUMMARY
Introduction/Objective Primary aim of malocclusion treatment is to achieve optimal occlusion. The purpose of this pilot prospective clinical study was to investigate digital occlusal features pre and post orthognathic surgery among individuals with skeletal class III malocclusion.
Methods Nine patients confirmed to have skeletal class III malocclusion participated in this study, including four females and five males. Single bite scan was performed seven days before and 6–8 weeks after surgery using T-Scan Novus (Tekscan Inc., Norwood, MA, USA). Occlusal time, total average occlusal force of upper and lower arch and projection of center of force (COF) were assessed and compared before and after surgery.
Results Compared to the pre-surgical period; occlusal time was significantly reduced ($p = 0.011$). While the total force in the anterior segment increased ($p = 0.008$), a decrease was noted in the posterior segment ($p = 0.008$). The COF demonstrated a markedly improved position in the post-surgical period, indicating a more stable occlusion ($p = 0.026$).
Conclusion In subjects with skeletal class III malocclusion, surgical treatment significantly improves occlusal quality, including occlusion and disclusion times, the distribution of total force in the antero-posterior direction, and the COFs, compared to the pre-surgical condition.
Keywords: T-Scan; skeletal class III; orthognathic surgery; digital occlusal analyzer

INTRODUCTION

The primary aim of malocclusion treatment is to achieve optimal occlusion, regardless of the presence of skeletal discrepancies. The development of dental occlusion is under the influence of functional and genetic factors, while in certain cases, malocclusion arises due to adaptations of masticatory muscles and temporomandibular joint during dental arch formation [1]. Premature occlusal contacts on teeth can induce dental stress, which in turn may contribute to pathological alterations in the supporting periodontal structures, temporomandibular joint complex, and the masticatory musculature [2]. Orthodontic therapy corrects malocclusion through precise alignment of the dentition and the establishment of an optimal interarch relationship, thereby achieving normal occlusion as characterized by the criteria of Ricketts, Roth, Andrews, and Angle [1, 3]. Orthognathic surgery is indicated in cases involving skeletal deformities. The primary goal of orthodontic treatment is to achieve optimal oral health by establishing proper aesthetics, function, and stability. A stable centric occlusion and maximal intercuspation serve as key indicators of a well-established and functional occlusion [1]. Following post-surgical orthodontic treatment and orthognathic surgery in patients with discrepancies in skeletal structure, it is crucial to establish reliable occlusal contacts that promote a balanced and uniform transmission of masticatory forces throughout the mandible [4, 5].

Occlusal relations may be evaluated through a range of occlusion analysis instruments, with articulating paper serving as the primary method for precisely identifying contact points between the upper and lower dental arches. However, while the articulating paper effectively records contact points, it cannot accurately assess the force applied at these points or quantify the strength of the occlusal loads generated [4, 5]. The T-Scan™ (Tekscan Inc., Norwood, MA, USA) computerized occlusal analysis system provides real-time occlusal contact force distribution and monitors dynamic changes from the initial tooth contact to the maximum intercuspal position during occlusion. This allows dental specialists to assess the location, force, and timing of occlusal contact using an ultrathin sensor foil, measuring around 0.1 mm (100 μm) in thickness. The center of force (COF) trajectory and the force-time graph are

Received • Примљено: July 23, 2025
Revised • Ревизија: October 6, 2025
Accepted • Прихваћено: October 10, 2025
Online first: October 22, 2025

Correspondence to:
Amar ĐERLEK
Mladena Mitrića 12/11
11030 Belgrade
Serbia
amardjerlek@gmail.com

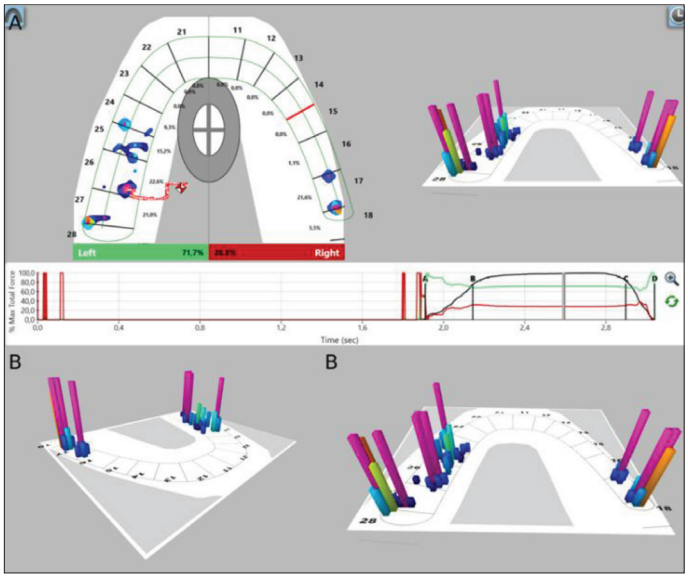


Figure 1. A – two-dimensional occlusal load interpretation; B – tree-dimensional occlusal load interpretation

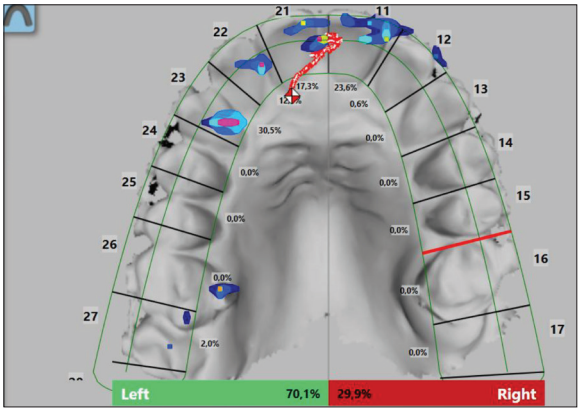


Figure 2. Three-dimensional occlusal load interpretation with imported stereolithography file

presented in two- and three-dimensional (2D, 3D) graphs. Digital data from the T-Scan™ (Tekscan Inc.) system allow accurate, focused modifications to achieve optimal occlusal balance. The dentition can also be divided into anterior and posterior regions, creating four analytical study units. There is also a possibility to upload stereolithography files from intraoral scans (Figures 1 and 2) [6, 7].

The aim of this pilot prospective clinical study was to assess changes in occlusal timing, total average occlusal force of the upper and lower arches, and the projection of COF in patients with skeletal class III malocclusion, pre- and post-orthognathic surgery.

METHODS

This prospective clinical pilot study was performed at School of Dental Medicine, University of Belgrade, Serbia. The clinical study involved nine consecutive participants treated at our clinic, all of whom fulfilled the predefined inclusion criteria. The sample included four females and five males, confirmed to have skeletal class III malocclusion who were treated with fixed orthodontic appliances, 0.018 inch slot, Roth prescription, with second molars included in both maxilla and mandible as preparation for orthognathic surgery. All patients underwent bimaxillary surgery. All participants provided written informed consent.

Digital occlusal parameters were recorded seven days before surgery and 6–8 weeks after (at the first orthodontic appointment) orthognathic surgery.

Inclusion criteria: skeletal class III malocclusion (ANB < 1°, Wits < 0 mm, patient may present discrepancies in either the vertical or transverse dimension); orthognathic surgical treatment; age (19–35 years).

Exclusion criteria: systemic diseases; temporomandibular disorders; bruxism; cleft lip and/or palate; craniofacial syndromes.

Three variables were assessed:

1. Occlusal time changes (time taken to go from initial contact to maximum intercuspation).
2. Total average occlusal force of the upper and lower arch withstanding at maximum intercuspation position.
3. Projection of COF before and after surgery illustrating the balance of occlusion.

Patients were seated in dentist's chair with a right angle (90°) between their upper and lower body. Data were recorded using the T-Scan™ (Tekscan Inc.) system. Prior to recording, the upper central incisor dimensions and tooth distributions were inserted into the T-Scan dental chart to personalize the graphical dental arches for more accurate occlusal arch mapping during measurements. Sensor calibration was performed to adjust its sensitivity level to the occlusal forces of each individual. Occlusal data were acquired using the T-Scan™ (Tekscan Inc.) system by recording contact points with a sensor foil. Data were carried out through a module known as the "handpiece", which was connected to a computer running processing software for visualizing the observed parameters (Figure 2 and 3).

Graphical interface is supported by the T-Scan software v 10 (Tekscan Inc.) [8, 9]. The sensor of the T-Scan™ system was placed in the mouth of the subject in the upright position, with its position guide located between the central incisors. Recording began by activating button on the handlebar. As parallel as possible to the occlusal plane, the sensor's handle was positioned. Each participant was instructed to occlude once onto the sensor foil applying maximal bite pressure for 1–2 seconds. Defined as the interval from first contact to maximum intercuspation, occlusal time was determined using the time tables provided by T-Scan software (version 10.0, Tekscan Inc.). A value of ≤ 0.2 seconds was regarded as ideal for occlusion time, and ≤ 0.4 seconds for disclusion time [10, 11, 12].

The T-Scan™ (Tekscan Inc.) system calculates relative force percentages for each tooth based on the total occlusal force at a given moment. These values are presented alongside timing data in seconds and are visualized through the force outliers tab in the timing table [11]. In the maximum intercuspation position, occlusal time, COF position,

Figure 3. T-Scan Novus (Tekscan Inc., Norwood, MA, USA) handpiece with sensor film

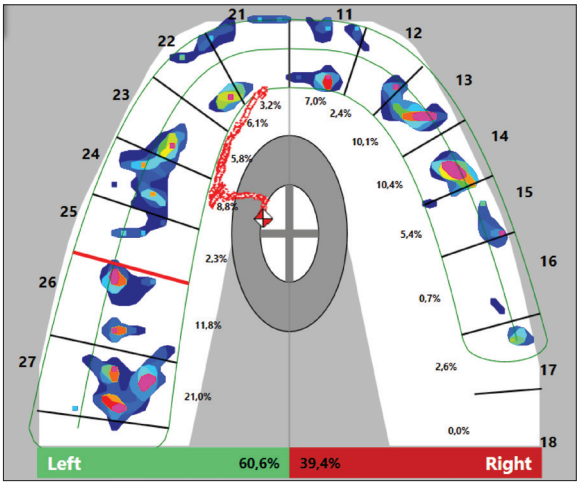


Figure 4. Presentation of center of force by the red and white diamonds in the two-dimensional graph

and force distribution percentages in the right-left (RL) and antero-posterior (AP) areas were evaluated. Values of the three readings were assessed for each patient. Force distribution percentages in the RL and AP regions were calculated by splitting the dental arch into four sections: anterior right, anterior left, posterior right, and posterior left. Percentages of anterior force were obtained from the central incisors to the canines, including the lateral incisors [13].

In the 2D graph, the COF is depicted using red and white diamonds (Figure 4), illustrating the balance of occlusion. It is used to establish proper stability of occlusion [11]. The COF locations were classified into three patterns: ideal, where the COF appears as a white point within the center of the ellipse; good, when it is located within the silver area of the outside ellipse; if it falls outside both designated zones, it is classified as outside of area. To assess closure stability, the trajectory of COF from initial tooth contact to maximum intercuspation was classified into four separate patterns: First – trajectory was considered normal when it proceeded in a direct line from the front to the back of the arch, roughly following the midline. Second – when the trajectory localized within a defined region instead of following a straight-line course, it was considered a contact point. Third – slipping in left-right, described as the trajectory, was delineated horizontally across the midline. Fourth – slipping in RL, described as the trajectory, was delineated horizontally relative to the midline [1] (Figure 5).

Using IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY, USA), the data were processed. To summarize the data, descriptive statistics was applied, including calculations of the mean, standard deviation (SD), minimum, and maximum values for each variable. In order to assess the evidence of statistically significant differences between paired (related) variables, the Wilcoxon signed-rank test was applied. Due to the non-normal distribution of the data, that test was chosen, as determined by preliminary assessments. The results were presented with corresponding p-values, statistical significance was regard at a p-value of less than 0.05. Statistical tests for all analyses were two-tailed.

Ethics: This study was performed at School of Dental Medicine, University of Belgrade, Belgrade, Serbia. Ethical approval was obtained from the institutional ethics committee (approval No. 36/34), and was carried out in line with the ethical standards described in the Declaration of Helsinki for studies involving human subjects.

RESULTS

The results present the mean ($\bar{x}$), SD, median, and minimum–maximum values for occlusal time and total average occlusal force. These results showed that patients with skeletal class III have a statistically significant improvement in occlusal time ($p = 0.011$) following orthognathic surgery, whereas disclusion time increased (0.28 ± 0.41 seconds) compared to the pre-surgical condition (0.27 ± 0.27 seconds) (Table 1).

The results for total average occlusal force show that there was a statistically significant difference in force distribution in both the anterior and posterior segments of the dental arch. The anterior segment shows an increase in total force after the surgical treatment, as seen in Table 1 (before 0.20 ± 0.11%, after 0.55 ± 0.18%), while the posterior segment shows a decrease in total force compared to the pre-surgical condition (before 0.80 ± 0.11%, after 0.45 ± 0.18%).

In contrast to the anterior and posterior segments, the left and right sides of the dental arch did not show statistically significant variation in force distribution in relation to the pre-surgical condition. However, it can be noted that the left side of the dental arch showed a decrease in total bite force after the intervention (before 0.55 ± 0.09%, after 0.48 ± 0.09%), while an increase in bite force was assessed on the right side (before 0.45 ± 0.09%, after 0.52 ± 0.10%).

COF is explained as a diamond-shaped icon. During data recording before surgery, patients exhibited various positional variations of the diamond-shaped icon, as shown in Table 1. All four possible trajectories, previously explained and illustrated in Figure 5, were present. After surgery, the COF tended to be more centralized, and all examined patients indicated a statistically significant difference ($p = 0.026$) in the position of COF. Its post-surgical position was within normal limits (Table 2).

Table 1. Descriptive statistics for occlusal time (seconds) and total average occlusal force (%)

Observed parameters	Before surgery x̄±SD Med; min–max	After surgery x̄±SD Med; min–max	Significance
Occlusion time	2.11 ± 0.76 1.7; 1.48–3.66	0.96 ± 0.64 0.75; 0.50–2.21	p = 0.011*
Disclusion time	0.27 ± 0.27 0.21; 0.00–0.96	0.28 ± 0.41 0.17; 0.05–1.37	p = 0.593
Total anterior occlusal force	0.20 ± 0.11 0.15; 0.06–0.35	0.55 ± 0.18 0.53; 0.37–0.93	p = 0.008*
Total posterior occlusal force	0.80 ± 0.11 0.85; 0.65–0.93	0.45 ± 0.18 0.47; 0.07–0.63	p = 0.008*
Total left occlusal force	0.55 ± 0.09 0.56; 0.36–0.64	0.48 ± 0.09 0.48; 0.37–0.71	p = 0.173
Total right occlusal force	0.45 ± 0.09 0.43; 0.36–0.63	0.52 ± 0.10 0.52; 0.28–0.63	p = 0.138

*Statistical significance

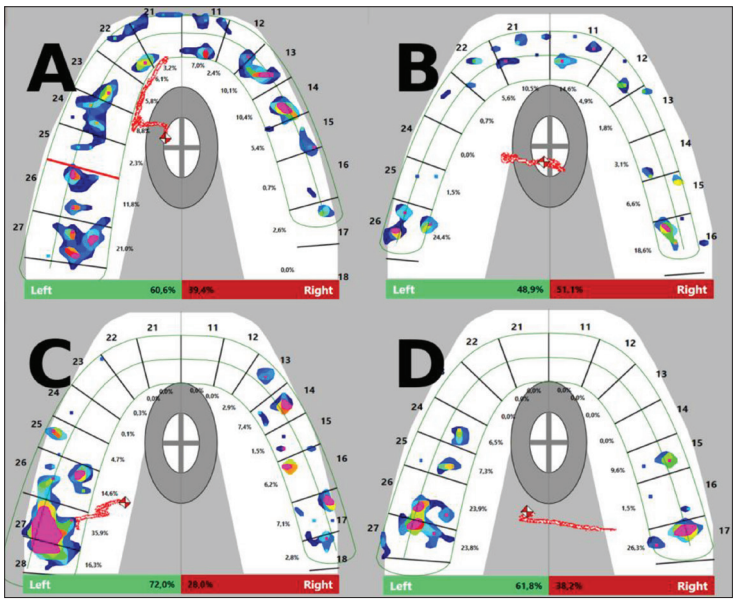


Figure 5. Four types of trajectories of the closing path: A – normal: the trajectory was straight-down in shape; B – contact point: the trajectory was clustered into a specific area; C – sliding in left-right, defined as the trajectory, was outlined horizontally across the midline; D – sliding in right-left: the trajectory was outlined horizontally, across the midline

Table 2. Descriptive statistics of location of the center of force before and after surgery

Center of force	Before surgery	After surgery	Significance
normal	3 (33.3%)	9 (100%)	p = 0.026*
L→R	3 (33.3%)	/	
R→L	2 (22.2%)	/	
contact	1 (1.1%)	/	

*Statistical significance; L – left; R – right; contact – trajectory localized in defined region

DISCUSSION

This pilot study examined occlusal factors in participants confirmed to have skeletal class III malocclusion, seven days preoperatively and 6–8 weeks postoperatively. Previous studies have investigated occlusion in patients with various occlusal classes at two or three different time points [14, 15, 16]. Digital devices enable improved accuracy and enhanced precision in analyzing the timing and intensity of occlusal contacts, while the T-Scan™ (Tekscan Inc.) system enables accurate assessment of a patient's bite [2, 17]. This technology also captures objective occlusal patterns during jaw movement, which can be crucial for effective orthodontic treatment. Regular monitoring allows for early detection and correction of occlusal discrepancies and related musculoskeletal imbalances [18, 19, 20]. Consequently, assessing occlusion both pre- and post-surgery, as demonstrated in this study, proves to be highly beneficial for patient outcomes.

Occlusal time gradually decreased after surgery, approaching the ideal physiological range. These findings suggest that minimizing occlusal time is important for facilitating smooth mandibular closure into maximum intercuspation without premature contacts. In contrast, disclusion time was within the physiological normal range both prior to and following the surgical intervention, with our findings indicating only minimal variations that did not reach statistical significance. Previous studies have reported that ideal physiological occlusal times is less than 0.2 seconds, with some authors suggesting a typical range between 0.1 and 0.4 seconds [1, 13]. The results of our research are consistent with those reported by Tammataratarn et al. [1]. Evidence from multiple studies by Ellis et al. [21] reported that individuals scheduled for orthognathic surgery due to dentofacial deformities exhibited lower maximum bite force compared to healthy controls, emphasizing the clinical relevance of evaluating masticatory strength prior to surgical intervention. Similar findings are also reported by Iwase et al. [22], who showed that their patients had a decreased forces postoperatively, compared to presurgical bite force. Results for total average occlusal force showed a statistically significant improvement in force distribution in AP area. Specifically, there was a notable increase in force distribution in the anterior segment after surgery, while a decrease in total occlusal force is observed in the posterior segment. As a result of the surgical intervention, subjects with skeletal class III malocclusion achieve proper incisal overjet, which allows the inclusion of a greater number of anterior teeth and a beneficial force distribution compared to the preoperative state [22].

In contrast to the AP segment, RL areas did not show statistically significant variations in force distribution after surgical procedure. This outcome may depend on several factors, such as the surgeon's skill, tooth positioning, or potential postoperative relapse. Compared to our study, Tammataratarn et al. [1] did not find a statistically significant variation between the experimental and control

groups in the AP and RL areas. However, they did observe a significant difference in bite force between the preoperative period and one month after surgery.

Postoperative assessments revealed improved force distribution and occlusal force transmission, this finding was consistent with the resulting trajectory course. The COF is a very important parameter that indicates the stability of a patient's occlusion [23, 24]. Analysis of the preoperative condition revealed a wide range of movement of the diamond shaped icon, indicating that most patients had highly unstable occlusion, with the exception of one patient who showed a consistent contact relationship (Table 2). Surgical intervention led to a significant improvement at the stability of occlusion in patients with skeletal class III malocclusion, which can be attributed to the applied combined orthodontic-surgical treatment [21]. Other authors have also reported findings consistent with ours, emphasizing that their patients exhibited a more centric COF [1].

This study is limited by a modest sample size and a short-term follow-up, considering that these patients typically undergo two or three years of combined orthodontic-surgical treatment. Future research will focus on expanding the variables being observed, such as standard deviations of force on individual teeth, including and monitoring a larger number of these patients across different time points and various phases of therapy, which may provide insight into the improvement or deterioration of the evaluated parameters, and consequently, the enhancement or decline in occlusal stability.

CONCLUSION

Surgical therapy significantly improves occlusal quality among individuals with skeletal class III malocclusion. It has been proven that it leads to improvements in occlusion time, the distribution of total force in AP direction, projection of COF, compared to the pre-surgical condition. The results have shown that further investigation is needed into distribution of force on the right and left regions, expanding the variables being observed, such as standard deviations of force on individual teeth in order to enhance the occlusal stability in these patients.

ACKNOWLEDGMENT

This paper is a part of Dr. Amar Đerlek's doctoral thesis.

Conflict of interest: None declared.

REFERENCES

1. Tammataratarn P, Chen MC, Lin CH, Wen-Ching Ko. Sequential digital occlusal analysis in patients with skeletal Class III malocclusion with orthognathic surgery: Surgery-first vs orthodontic-first approach. Am J Orthod Dentofacial Orthop. 2022;162(6):927–36. [DOI: 10.1016/j.ajodo.2021.07.030] [PMID: 36163310]
2. Chowdhary R, Sonnahalli NK. Clinical applications of the t-scan quantitative digital occlusal analysis technology asystematic review. Int J Comput Dent. 2024;27(1):49–86. [DOI: 10.3290/j.ijcd. b3945153] [PMID: 36928754]
3. Manikandhan R, Manikandan S, Ponvel K, Parameswaran R, Shankar S. Comparison of Occlusal Digital Analysis Using T-Scan in Orthognathic Surgery with Only Conventional Orthodontic Patients. J Maxillofac Oral Surg. 2023;22(2):397–409. [DOI: 10.1007/s12663-023-01862-2] [PMID: 37122794]
4. Li DTS, Leung YY. Temporomandibular Disorders: Current Concepts and Controversies in Diagnosis and Management. Diagnostics (Basel). 2021;11(3):459. [DOI: 10.3390/diagnostics11030459] [PMID: 33800948]
5. Bunpu P, Changsiripun C. Assessment of masticatory performance in patients undergoing orthognathic surgery: A systematic review and meta-analysis. J Oral Rehabil. 2023;50(7):596–616. [DOI: 10.1111/joor.13447] [PMID: 36920311]
6. Komuravelli A, Gawande S, Katekhaye S, Bhoir P. Occlusal indicators-a simplified approach. J Interdiscip Dent Sci. 2021;10(1):7–14.
7. Qadeer S, Özcan M, Edelhoff D, Van Pelt H. Accuracy, reliability and clinical implications of static compared to quantifiable occlusal indicators. Eur J Prosthodont Restor Dent. 2021;29(3):130–41. [DOI: 10.1922/EJPRD_2202Qadeer12] [PMID: 33508180]
8. Wang Q, Zhao Z, Li J, Zhou M, Tang K, Bai S, et al. In vivo evaluation of T-Scan in quantifying occlusal contact. J Oral Rehab. 2024;51(9):1675–83. [DOI: 10.1111/joor.13732] [PMID: 38926933]
9. Reich KM, Tatzber V, Skolka A, Piehslinger E, Lettner S, Kundi M, et al. A comparative study of digital and conventional occlusal indicators: accuracy and reliability of the T-Scan Novus, wax occlusogram, and articulating silk in clinical application. J Dent. 2025;156:105695. [DOI: 10.1016/j.jdent.2025.105695] [PMID: 40096877]
10. Prasanthi GS, Sajjan MCS, Manikyamba YJB, Chandrasekhar PVB, Kotemsetty SL. An analysis of three-dimensional changes to comprehensively evaluate the occlusion time, disclusion time, point of first contact and force distribution at various head positions using Tekscan III: An in vivo study. J Indian Prosthodont Soc. 2024;24(4):341–50. [DOI: 10.4103/jips.jips_51_24] [PMID: 40227939]
11. Tekscan Inc. T-Scan™ Novus User Manual. South Boston, MA, USA: Tekscan Inc, 2022. Available from: https://api.klockner.es/assets/fichas_tecnicas/T-Scan-User-Manual-10.0x-(RevW).pdf
12. Basson E, Kerstein R, Radke J. Ability to correctly select high force occlusal contacts from articulating paper markings. Adv Dent Technol. 2020; 2:101–10.
13. Iyer PK, Venugopalan S, N T, Jebaraj S. T-scans in implant procedures. Bioinformation. 2023;19(1):35–8. [DOI: 10.6026/97320630019035] [PMID: 37720290]
14. Maurya RK, Bhardwaj P, Singh H, Mitra R, Nakra O, Mishra HA. Evaluation of pre- and post-treatment masticatory and bite force efficiency in patients undergoing fixed orthodontic treatment and orthognathic surgery using T-Scan Novus occlusal analysis: A factorial randomized controlled trial. Natl J Maxillofac Surg. 2022;13(3):376–83. [DOI: 10.4103/njms.njms_48_22] [PMID:36683942]
15. Hatab N, Abutayyem H, Al Dwiri OHE, Rafaa A, Ivanjac F. Significance of T-ScanTM in recording occlusion parameters in orthodontic patients. Srp Arh Celok Lek. 2025;153(1–2):10–16. [DOI: 10.2298/SARH241125013H]
16. Giray B, Sadry S. Modifications in Class I and Class II Div. 1 malocclusion during orthodontic treatment and their association with TMD problems. Cranio. 2021;39(1):65–73. [DOI: 10.1080/08869634.2019.1572282] [PMID: 30741126]
17. Manziuc MM, Savu MM, Almășan O, Leucuța DC, Tăut M, Ifrim C, et al. Insights into Occlusal Analysis: Articulating Paper versus Digital Devices. J Clin Med. 2024;13(15):4506. [DOI: 10.3390/jcm13154506] [PMID: 39124772]
18. Toser I, Faur AB, Anghel-Lorinți AE, Jivănescu A. Case Report: A comprehensive digital workflow to enhance predictability and precision with fixed dental prostheses in the posterior region. Front Dent Med. 2025;6:1625405. [DOI: 10.3389/fdmed.2025.1625405] [PMID: 40630263]

19. Rubió-Ferrer G, Rovira-Lastra B, Khoury-Ribas L, Flores-Orozco EI, Ayuso-Montero R, Martinez-Gomis J. Reference values and reliability of occlusal force distribution and occlusal time measured by the T-Scan system in adults with healthy dentition. J Prosthodont. 2024;33(6):558–64. [DOI: 10.1111/jopr.13838] [PMID: 38469973]
20. Rovira-Lastra B, Khoury-Ribas L, Flores-Orozco EI, Ayuso-Montero R, Chaurasia A, Martinez-Gomis J. Accuracy of digital and conventional systems in locating occlusal contacts: A clinical study. J Prosthet Dent. 2024;132(1):115–22. [DOI: 10.1016/j.prosdent.2023.06.036] [PMID: 37612195]
21. Ellis E 3rd, Throckmorton GS, Sinn DP. Bite forces before and after surgical correction of mandibular prognathism. J Oral Maxillofac Surg. 1996;54(2):176–81. [DOI: 10.1016/s0278-2391(96)90443-7] [PMID: 8604066]
22. Iwase M, Sugimori M, Kurachi Y, Nagumo M. Changes in bite force and occlusal contacts in patients treated for mandibular prognathism by orthognathic surgery. J Oral Maxillofac Surg. 1998;56(7):850–5; discussion 855–6. [DOI: 10.1016/s0278-2391(98)90013-1] [PMID: 9663576]
23. Yang L, Zhou T, Zhao N, Qu X, Chen W, Zhao Y, et al. [The impact of oral occlusal exercises on occlusal performance of oral cancer patients following segmental mandibulectomy without simultaneous reconstruction]. Shanghai Kou Qiang Yi Xue. 2025;34(2):184–90. [Article in Chinese] [DOI: 10.19439/j.sjos.2025.02.013] [PMID: 40550772]
24. Abe KI, Sakaguchi K, Mehta NR, Correa LP, Abdallah EF, Yokoyama A. Effect of body posture on stability and balance of occlusal contacts. Cranio. 2025;43(2):225–35. [DOI: 10.1080/08869634.2022.2119301] [PMID: 36101939]

Утицај ортогнатске хирургије на оклузалне контакте и дистрибуцију силе загрижаја код пацијената са III скелетном класом применом система T-скен

Амар Ђерлек[1], Неда Стефановић[1], Зорана Стаменковић[1], Тина Пајевић[1], Иван Арсић[1], Биљана Миличић[2], Ненад Недељковић[1]

[1]Универзитет у Београду, Стоматолошки факултет, Клиника за ортопедију вилица, Београд, Србија;
[2]Универзитет у Београду, Стоматолошки факултет, Медицинска статистика и информатика, Београд, Србија

САЖЕТАК

Увод/Циљ Примарни циљ лечења малоклузије је постизање оптималне оклузије. Сврха ове проспективне клиничке пилот-студије била је да се процене дигитални оклузални параметри пре и после ортогнатске хирургије код пацијената са III скелетном класом.

Методе Студија је обухватила девет пацијената – четири особе женског и пет мушког пола, код којих је дијагностикована малоклузија III скелетне класе. Скенирање једног загрижаја извршено је седам дана пре и шест до осам недеља након хирургије, користећи T-скен Новус (*Tekscan Inc*., Норвуд, МА, САД). Време оклузије, укупна просечна оклузална сила горњег и доњег зубног лука и пројекција центра силе процењени су и упоређени пре и после операције.

Резултати У поређењу са преоперативним периодом, време оклузије било је значајно смањено ($p = 0,011$). Док је укупна сила у предњем сегменту порасла ($p = 0,008$), у задњем сегменту је забележено њено смањење ($p = 0,008$). Центар силе је показао значајно бољу позицију у постоперативном периоду, што указује на стабилнију оклузију ($p = 0,026$).

Закључак Код пацијената са III скелетном класом, хируршка терапија значајно побољшава квалитет оклузије укључујући време оклузије и дисклузије, расподелу укупне силе у антеро-постериорном правцу, као и центар силе, у поређењу са преоперативним стањем.

Кључне речи: T-скен; III скелетна класа; ортогнатска хирургија; дигитални оклузални анализатор

DOI: https://doi.org/10.2298/SARH251109094D
UDC: 616.833.15-009.7:616.89-008.454-056.24

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Association between depression and oro-facial pain – a pilot study

Igor Đorđević[1], Filip Ivanjac[2], Danica Popović Antić[1], Minja Miličić Lazić[1], Momčilo Čolić[1], Luka Župac[1]

[1]University of Belgrade, School of Dental Medicine, Clinic for Prosthodontics, Belgrade, Serbia;
[2]University of Belgrade, School of Dental Medicine, Clinic for Maxillofacial Surgery, Belgrade, Serbia

SUMMARY

Introduction/Objective Orofacial pain and depression are frequent symptoms when temporomandibular dysfunction (TMD) is present. The aim of this prospective study was to evaluate the influence of pain intensity on the level of depression in patients with TMD.

Methods This study included 44 patients, heterogeneous in sex and age from 25 to 45 years. Subjects were evaluated for signs of painful TMD based on the Research Diagnostic Criteria for TMD protocol. The study protocol was composed of a combination of data on clinical signs, a pain scale numerical, visual analogue scale, and a depression related questionnaire symptoms check list (SCL-90R).

Results When assessed the type of dysfunction and degree of the depression no statistically significant differences were found (p = 0.420). No statistically significant difference was observed in the age of respondents without depression, with moderate and severe depression (p = 0.859), There was no statistically significant difference observed in the degree of chronic pain in patients without depression and patients with a moderate and severe form of depression (p = 0.119). Pain on a numerical scale did not differ significantly in relation to the occurrence and degree of depression (p = 0.171; p = 0.068; p = 0.091). However, there was a statistically significant difference in the psychosocial status between the respondents in relation to the degree of depression regarding social life and daily activity (p = 0.010; p = 0.002).

Conclusion Depression can be one of the causes, but also a consequence of chronic oro-facial pain, and thus indirectly a factor that affects the psychosocial state and quality of life of the patients.

Keywords: depression; orofacial pain; temporomandibular dysfunction

INTRODUCTION

Orofacial pain is an unpleasant sensory and emotional experience associated with immediate or potential tissue damage. The most common cause of pain in the oro-facial region of non-odontogenic origin are musculoskeletal disorders involving the muscles of the cervical spine, temporomandibular joints, and masticatory musculature [1, 2, 3]. Although they do not jeopardize vital functions, musculoskeletal disorders significantly affect the quality of life [4, 5]. Patients with oro-facial pain are challenging for a therapist due to the complexity of the symptoms and signs they present, as well as the diversity of clinical forms of pain in the face and jaw region. Precisely for the above reasons, it was believed that myalgias from arthralgias originate from the same cause. Disorders involving the structures of the temporomandibular joint and masticatory muscles are classified as temporomandibular dysfunction (TMD) [6, 7, 8].

Depression, fear, anxiety, physical damage, and disability caused by pain further complicate the existing condition, decreasing the patients' quality of life. Although depression is a common symptom in patients with chronic orofacial pain, or chronic pain in general, there is no evidence that depression or any psychological disorder causes TMD symptoms. The claim that pain precedes depression is more likely [9, 10].

The aim of this prospective study was to evaluate the influence of pain intensity on the level of depression in patients with TMD.

METHODS

The research was conducted as a prospective study that included 44 patients, heterogeneous in terms of sex and age. The sample was selected of patients who came to the Clinic for Prosthodontics of the School of Dental Medicine, University of Belgrade with some of the symptoms and signs of TMD. Subjects that were evaluated to have signs of painful TMD based on the Research Diagnostic Criteria for TMD (RDC/TMD) protocol (Dworkin and LeResche) Appendix 1, were in an age range from 25 to 45 years.

The exclusion criteria:

1. Patients with the pain of other origin: odontogenic, neurogenic, vascular, inflammatory, or related to tumor changes in the surrounding structures (ear, throat, eye, nose, sinuses);
2. Patients who had some other chronic disease that impairs the general health condition and gives a false image of TMDs;

Received • Примљено: November 9, 2025
Accepted • Прихваћено: December 8, 2025
Online first: December 10, 2025

Correspondence to:
Filip IVANJAC
Svetogorska 18
11000 Belgrade
Serbia
filipivanjac@yahoo.com

3. patients younger than 25 and older than 45 years;
4. patients who did not consent to participate in the study.

A detailed clinical examination of the orofacial system was performed in all subjects in order to determine the presence of symptoms and signs of TMD. Patients with symptoms and signs of TMD were included in the study. All subjects were healthy and thoroughly informed about the research protocol. Clinical examination and functional analysis of the oro-facial system were based on the diagnostic protocol RDC/TMD (Dworkin and LeResche, 1992), shown in Appendix 1, where one or more symptoms of painful muscle and/or joint dysfunction were recorded:

1. pain in the preauricular region;
2. pain or sensitivity when palpating the masticatory muscles;
3. limited and/or painful movements of the lower jaw;
4. deflection of the lower jaw during mouth opening;
5. presence of sound phenomena when opening the mouth.

The inclusion criteria for the study:

1. subjects with intact teeth;
2. subjects not surgically or orthodontically treated;
3. subjects that were not under medication therapy;
4. presence of painful symptoms in the region of the face and jaws.

The study protocol was composed of a combination of data on clinical signs, a pain scale numerical, visual analogue scale, and a depression related questionnaire symptoms check list (SCL-90R) (Appendix 2). For all respondents, the degree of chronic pain, the degree of depression (normal state, moderate depression, severe depression) was determined within the questionnaire (Axis II) Appendix 2.

In the list of questions Axis II related to pain and the psychosocial status of the subjects, data were obtained based on the subjects' answers to the questions offered in the RDC/TMD protocol.

The Axis II list includes:

1. a short questionnaire of seven questions for evaluating the intensity of pain (0–4);
2. SCL-90 for expressing the degree of depression (normal state, moderate depression, severe depression);
3. the offered list of 10 functions of the orofacial system that are most often difficult to perform (it is necessary to determine the number of positive answers in relation with offered functions).

Pain intensity was expressed by values from 0 to 100, which were calculated by multiplying the mean value obtained from the answers to questions 7, 8 and 9 by 10. In response to these questions, the respondent was asked to choose a value on the offered numerical scale from 0 to 10: mean value (current pain, worst pain experienced in the past six months, average pain in the past six months) × 10, changes in social contact were expressed in values from 0 to 100 and were the result of answers to questions 11, 12, 13. The respondent chose the value on the offered numerical scale from 0 to 10 mean value (daily activities, social activities) × 10, 0–29 = 0 points, 30–49 = 1 point, 50–69 = 2 points, 70 and more = 3 points. After the evaluation is completed and the results expressed in points are added up, chronic pain is expressed: Grade 0 – pain due to TMD has not been present in the last six-month-period. Grade I – low pain intensity (pain intensity less than 50) and slightly changed social contacts and (less than 3 points). Grade II – high pain intensity (greater than 50) and altered social contacts (more than 3 points).

To calculate the degree of depression, the answers to the questions in the questionnaire were used, expressed in numerical equivalents, and then summed up. The total was divided by the number of responses requested. If the obtained value was less than 0.535, depression was not present. In the interval from 0.535 to 1.105 the depression was moderate. A value greater than 1.105 indicated severe depression. The statistical software package SPSS for Windows (18.0) (SPSS Inc., Chicago, IL, USA) was used for data processing. At the beginning of the research, all variables were described using classic descriptive methods. Attributive features are described by absolute and relative numbers, and numerical measures of central tendency (arithmetic mean and median) and variability measures (standard deviation, minimum, and maximum value), as well as 95% confidence interval. The choice of tests for the analysis of numerical features of observation depended on the nature of their distribution, which was examined using the Koglomorov–Smirnov test. In the case of a normal distribution of data and testing the difference between more than two groups of subjects, a one-factor analysis of variance was used, while for non-parametric data the Kruskal–Wallis test was used. The threshold value for accepting the working hypothesis was set at $p < 0.05$.

Ethics: This study was approved by the Ethics Committee of the School of Dental Medicine, University of Belgrade by decision number 36/6.

RESULTS

Between the analyzed subpopulations of TMD, no statistically significant difference was observed in the severity of depression recorded during the examination (Table 1).

In the group of subjects with musculo-articular dysfunction, most subjects showed symptoms of moderate depression. Most of the respondents showed a moderate form of depression, regardless of the type of dysfunction. Excluding the degree of expression, depression is most

Table 1. Level of depression relation with dyagnosis

Depression		Diagnosis			Significance
		Muscular dysfunction	Articular dysfunction	Muscular and articular dysfunction	
Level of depression n (%)	None	1 (14.3%)	8 (53.3%)	7 (31.8%)	p = 0.420
	Moderate	5 (71.4%)	5 (33.3%)	11 (50%)	
	Severe	1 (14.3%)	2 (13.3%)	4 (18.2%)	

*statisticaly significant difference

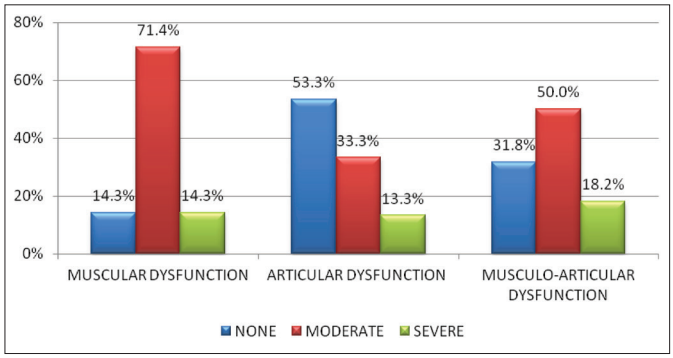


Figure 1. Dysfunction and degree of depression

Table 2. Adulthood of the subjects related to the level of depression

Participant adulthood		Mean	Med	SD	Min	Max	95% CI
Age	No depression	39.06	39.50	8.378	23	54	34.60–43.53
	Moderate depression	38.33	36.00	12.16	22	55	32.80–43.87
	Severe depression	36.29	30.00	13.24	21	53	24.04–48.53

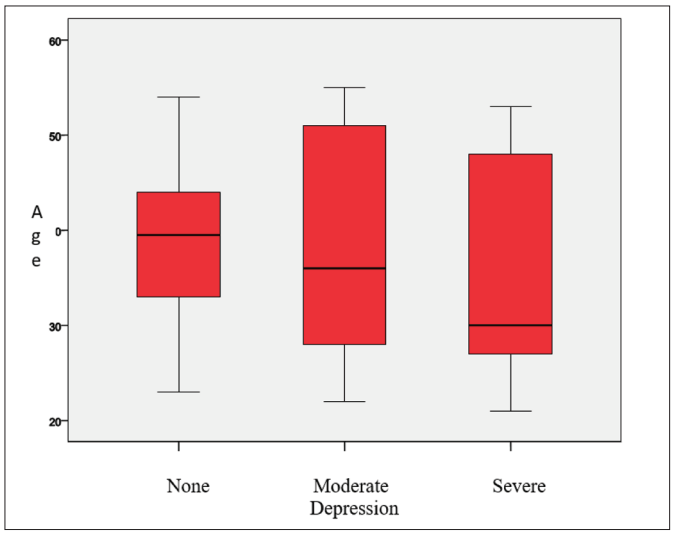


Figure 2. Age of the participants and the level of depression

Table 3. Level of chronical pain related to the level of depression

Observed parameters		Mean	Med	SD	Min	Max	95% CI
Level of chronical pain	No depression	1.44	1.00	0.512	1	2	1.16–1.71
	Moderate depression	1.76	2.00	0.436	1	2	1.56–1.96
	Severe depression	1.71	2.00	0.488	1	2	1.26–2.17

prevalent in the group of respondents with signs of muscle dysfunction (Figure 1).

No statistically significant difference was observed in the age of respondents without depression, with moderate and severe depression (one-factor analysis of variance; p = 0.859), Table 2).

The age of the participants and the level of depression are presented in Figure 2.

The level of chronical pain related to the level of depression are presented in Table 3.

There was no statistically significant difference observed in the degree of chronic pain in patients without depression and patients with a moderate and severe form of depression (Table 4).

Pain intensity expressed on a numerical scale according to the degree of depression are presented in Table 5.

Severity of pain on a numerical scale and depression are presented in Figure 3.

Pain, measured by a numerical scale, did not differ statistically significantly in relation to the occurrence and degree of depression (Table 6).

Social life and depression are presented in Figure 4.

Daily activity and depression are presented in Figure 5.

There was a statistically significant difference in the psychosocial status between the respondents in relation to the degree of depression (Table 8).

DISCUSSION

The influence of psychogenic factors in the occurrence and development of temporomandibular disorders is particularly significant. Nevertheless, chronic pain can affect the patient's psychosocial status and thus worsen the existing condition. Masticatory muscles react to induced stress with increased activity, and their response to stress is significantly more intense than in other skeletal muscles [11–14].

Depression is a frequent companion of chronic pain conditions and it has been documented in the clinical population of TMDs. The International Classification F32 defines depression as a disease accompanied by a bad mood, a decrease in activity and vital energy, expressed in mild, moderate, and severe depressive episodes [14, 15, 16].

Given that psychological disorders due to painful sensations are frequent companions of chronic as well as acute pain in the orofacial region, a shorter informative conversation between the therapist and the patient is necessary on the basis of which the primary assessment of psychological and psychosocial ability is performed. As an integral part of the evaluation of TMD patients, an examination and determination of psychological status should be included in order to detect symptoms of depression and possibly predict the chronicity of TMD [15–18].

Analyzing the results of our research, in the group of subjects with muscular and articular dysfunction, most subjects showed symptoms of moderate depression, regardless of the type of dysfunction. In each and every degree of expression, depression is most prevalent in the group of respondents with signs of muscle dysfunction. Nevertheless, there were no statistically significant differences between the assessed groups. (Table 1, Figure 1). When we compared the severity of depression regarding the age of the patients there was no statistically significant difference observed in the age of respondents without depression, with moderate and severe depression (p = 0.859) (Table 2). The results show that the degree of chronic pain of the oro-facial system does not affect the expression of depression. When we compared the level of chronic pain – visual analogue scale, in relation with the level of depression, we used Kruskal–Wallis test to determine the significance of the assessed variables, there were no statistically significant differences observed in the degree of chronic pain in patients without depression and patients with a

Table 4. Level of chronical pain and depression

Observed parameters (X ± SD)	Depression			Significance[a]
	None	Moderate	Severe	
Level of chronical pain	1.44 ± 0.512	1.76 ± 0.436	1.71 ± 0.488	p = 0.119

*Statistically significant difference
[a]Kruskal–Wallis test

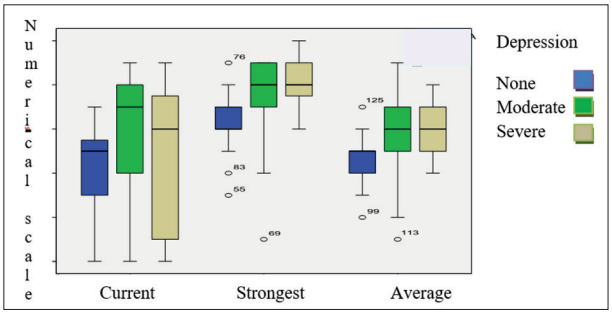


Figure 3. Severity of pain on a numerical scale and depression

Table 5. Pain intensity expressed on a numerical scale according to the degree of depression

Pain intensity		Mean	Med	SD	Min	Max	95% CI
Current pain	No depression	4.25	5.00	2.145	0	7	3.11–5.39
	Moderate depression	5.95	7.00	2.854	0	9	4.65–7.25
	Severe depression	4.57	6.00	3.823	0	9	1.04–8.11
Strongest pain	No depression	6.25	6.00	1.483	3	9	5.46–7.04
	Moderate depression	7.14	8.00	2.104	1	9	6.18–8.10
	Severe depression	8.14	8.00	1.345	6	10	6.90–9.39
Average pain	No depression	4.50	5.00	1.211	2	7	3.85–5.15
	Moderate depression	5.62	6.00	2.156	1	9	4.64–6.60
	Severe depression	6.00	6.00	1.414	4	8	4.69–7.31

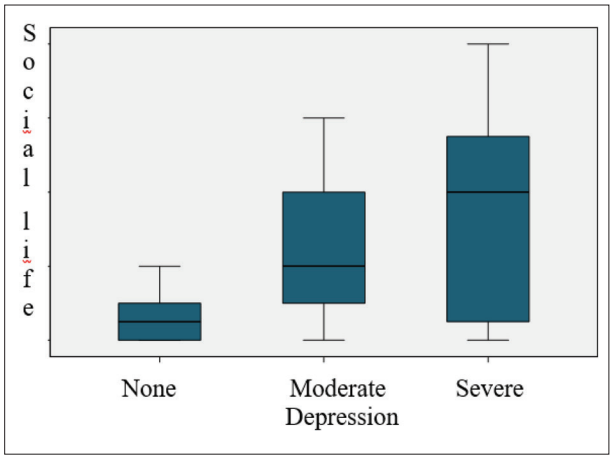


Figure 4. Social life and depression

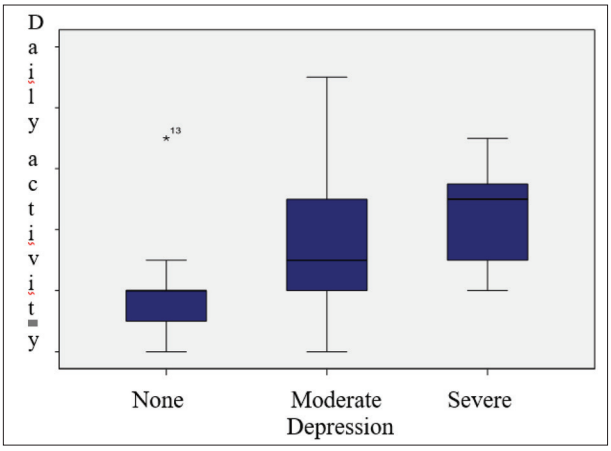


Figure 5. Daily activity and depression

Table 6. Subjectively assessed pain intensity with a numerical scale and depression

Intensity of pain (x ± sd)	Depression			Significance[a]
	None	Moderate	Severe	
Current pain	4.25 ± 2.145	5.95 ± 2.854	4.57 ± 3.823	p = 0.171
Strongest pain	6.25 ± 1.483	7.14 ± 2.104	8.14 ± 1.345	p = 0.068
Average pain	4.50 ± 1.211	5.62 ± 2.156	6.00 ± 1.414	p = 0.091

*statistical significance
[a]one-factor analysis of variance

Table 7. Psychosocial parameters expressed on a numerical scale according to the degree of depression

Psychosocial status		Mean	Med	SD	Min	Max	95% CI
Social life	No depression	0.56	0.50	0.629	0	2	0.23–.90
	Moderate depression	2.24	2.00	1.895	0	6	1.38–3.10
	Severe depression	3.43	4.00	3.155	0	8	0.51–6.35
Daily activity	No depression	1.88	2.00	1.628	0	7	1.01–2.74
	Moderate depression	3.62	3.00	2.148	0	9	2.52–4.72
	Severe depression	4.43	5.00	1.902	2	7	2.67–6.19

moderate and severe form of depression (Tables 3 and 4). When we assessed the level of pain registered on a numerical scale with the level of depression, it did not differ statistically significantly in relation to the occurrence and degree of depression (Tables 5 and 6). This was suggested by the other authors when they said that depression is independently associated with chronic oro-facial pain [2, 10]. Finally, when we wanted to assess the relation between psychosocial parameters like daily activity and social life, expressed on a numerical scale according to the degree of depression (Table 7) there was a statistically significant difference in the psychosocial status between the respondents in relation to the degree of depression ($p \leq 0.05$) (Table 8). Similarly, other authors have proven that TMD through subsequent depression significantly influenced patients' quality of life [5, 15]. In our study statistically significant difference was observed in

Table 8. Psychosocial status and depression

Observed parameters (X ± SD)	Depression			Significance[a]
	None	Moderate	Severe	
Social life	0.56 ± 0.629	2.24 ± 1.895	3.43 ± 3.155	p = 0.010*
Daily activity	1.88 ± 1.628	3.62 ± 2.148	4.43 ± 1.902	p = 0.002*

*Statistically significant difference
[a]Kruskal–Wallis test

the psychosocial status of the respondents in relation to the degree of depression. This means that the level of depression influenced the patients' daily activities as well as social life in terms of the intensity of the symptoms directly influencing the level of psychosocial parameters.

CONCLUSION

Depression can be one of the causes, but also a consequence of chronic oro-facial pain, and thus indirectly a factor that affects the psychosocial state and quality of life of the patients.

ACKNOWLEDGMENT

This study is part of PhD thesis of Prof. Dr Igor Đorđević.

Conflict of interest: None declared.

REFERENCES

1. Anita H, Asnely Putri F, Maulina T. The Association Between Orofacial Pain and Depression: A Systematic Review. J Pain Res. 2024;17:785–96. [DOI: 10.2147/JPR.S435219] [PMID: 38440413]
2. Karamat A, Smith JG, Melek LNF, Renton T. Psychologic Impact of Chronic Orofacial Pain: A Critical Review. J Oral Facial Pain Headache. 2022;36(2):103–40. [DOI: 10.11607/ofph.3010] [PMID: 35943323]
3. Shetty SS, de Arruda JAA. What is the impact on mental pollution and depression in patients with chronic orofacial pain? Spec Care Dentist. 2021;41(6):760–1. [DOI: 10.1111/scd.12619] [PMID: 34038597]
4. Holmström AK, Vallin S, Wänman A, Lövgren A, Stålnacke BM. Effect on orofacial pain in patients with chronic pain participating in a multimodal rehabilitation programme – a pilot study. Scand J Pain. 2023;23(4):656–61. [DOI: 10.1515/sjpain-2023-0004] [PMID: 37327054]
5. Tanner J, Teerijoki-Oksa T, Kautiainen H, Vartiainen P, Kalso E, Forssell H. Health-related quality of life in patients with chronic orofacial pain compared with other chronic pain patients. Clin Exp Dent Res. 2022;8(3):742–9. [DOI: 10.1002/cre2.560] [PMID: 35347879]
6. Nilsson IM, Ekberg E, Michelotti A, Al-Khotani A, Alstergren P, Conti PCR, et al. Diagnostic criteria for temporomandibular disorders-INfORM recommendations: Comprehensive and short-form adaptations for children. J Oral Rehabil. 2023;50(2):99–112. [DOI: 10.1111/joor.13390] [PMID: 36373958]
7. De la Torre Canales G, Poluha RL, Soares FFC, Ferreira DMAO, Sánchez-Ayala A, Bonjardim LR, et al. Who is the patient with resistant myofascial temporomandibular disorders pain? A somatosensory, psychosocial, and genetic characterization. J Headache Pain. 2025;26(1):98. [DOI: 10.1186/s10194-025-02055-7] [PMID: 40329183]
8. Neubert JK, Allen K, Alliston T, Almarza AJ, Athanasiou KA, Donertas-Ayaz B,et al. Preclinical perspectives on disorders of the temporomandibular joint: Tracing the past, navigating the present, and shaping the future. J Pain. 2025;36:105560. [DOI: 10.1016/j.jpain.2025.105560] [PMID: 40953762]
9. Alessandri-Bonetti A, Sangalli L, Boggero IA. Relationship between insomnia and pain in patients with chronic orofacial pain. Pain Med. 2024;25(5):319–26. [DOI: 10.1093/pm/pnae003] [PMID: 38258535]
10. De Sousa BM, López-Valverde A, Caramelo F, Rodrigues MJ, López-Valverde N. Use of antidepressants in the treatment of chronic orofacial pain caused by temporomandibular disorders: A randomized controlled clinical trial. Med Clin (Barc). 2024;163(2):74–7. [Article in English, Spanish] [DOI: 10.1016/j.medcli.2024.02.002] [PMID: 38570297]
11. Marchesi A, Sardella A, Khijmatgar S. Depression and Anxiety as Predictors of Pain and Sensory Thresholds in Adults with and Without Temporomandibular Disorder: A Case-Control Study. J Int Soc Prev Community Dent. 2025;15(3):257–64. [DOI: 10.4103/jispcd.jispcd_236_24] [PMID: 40838030]
12. Orzeszek S, Martynowicz H, Smardz J, Wojakowska A , Bombala W, Mazur G. et al. Assessment of sleep quality in patients with orofacial pain and headache complaints: A polysomnographic study. Dent Med Probl. 2024;61(4):549–62. [DOI: 10.17219/dmp/177008] [PMID: 38832763]
13. Delgado-Delgado R, Iriarte-Álvarez N, Valera-Calero JA, Centenera-Centenera MB, Garnacho-Garnacho VE, Gallego-Sendarrubias GM. Association between temporomandibular disorders with clinical and sociodemographic features: An observational study. Int J Clin Pract. 2021;75(5):e13961. [DOI: 10.1111/ijcp.13961] [PMID: 33387380]
14. Vučurević M, Vujović M, Stojanović-Tasić M, Marić N. Frequency and correlates of depression at the primary health care level in Belgrade. Srp Arh Celok Lek. 2020;148(3–4):185–90. [DOI: 10.2298/SARH190322098V]
15. Stanković Ž, Nikolić-Balkoski G, Leposavić Lj, Popović Lj. Perception of quality of life and social adjustment of patients with recurrent depression. Srp Arh Celok Lek. 2006;134(9–10):369–74. [DOI: 10.2298/SARH0610369S] [PMID: 17252901]
16. Leposavić I, Leposavić Lj. Attribution style of patients with depression. Srp Arh Celok Lek. 2009;137(9–10):529–33. [DOI: 10.2298/SARH0910529L] [PMID: 19950761]
17. Đorđević I, Lazić V, Todorović A, Čairović A, Veselinović V, Živković S. Transcutaneous electrical nerve stimulation therapy in reduction of orofacial pain. Srp Arh Celok Lek. 2014;142(3–4):150–4. [DOI: 10.2298/SARH1404150D] [PMID: 24839768]
18. Lončar J, Panić Z, Stojšin I, Savović S, Petrović B. Applicability of visual-analogue scale in patients with orofacial pain. Srp Arh Celok Lek. 2013;141(7–8):454–9. [DOI: 10.2298/SARH1308454L] [PMID: 24073550]

Повезаност депресије и орофацијалног бола – пилот-студија

Игор Ђорђевић[1], Филип Ивањац[2], Даница Поповић Антић[1], Мињa Миличић Лазић[1], Момчило Чолић[1], Лука Жупац[1]

[1]Универзитет у Београду, Стоматолошки факултет, Клиника за стоматолошку протетику, Београд, Србија;
[2]Универзитет у Београду, Стоматолошки факултет, Клиника за максилофацијалну хирургију, Београд, Србија

САЖЕТАК

Увод Орофацијални бол и депресија су чести симптоми када је присутна темпоромандибуларна дисфункција (ТМД). Циљ ове проспективне студије био је да се процени утицај интензитета бола на ниво депресије код пацијената са ТМД.

Методе Ова студија је обухватила 44 пацијента, хетерогених по полу и старости (од 25 до 45 година). Код испитаника је процењивано постојање знакова болне ТМД на основу протокола истраживачких дијагностичких критеријума за ТМД. Протокол студије се састојао од комбинације података о клиничким знацима, нумеричке скале бола, визуелне аналогне скале и упитника везаног за депресију – Листе за проверу симптома (*SCL-90R*).

Резултати Приликом процене врсте дисфункције и степена депресије нису пронађене статистички значајне разлике ($p = 0,420$). Није примећена статистички значајна разлика у старости испитаника без депресије, са умереном и тешком депресијом ($p = 0,859$). Није примећена статистички значајна разлика у степену хроничног бола код пацијената без депресије и пацијената са умереним и тешким обликом депресије ($p = 0,119$). Бол на нумеричкој скали није се значајно разликовао у односу на појаву и степен депресије ($p = 0,171$; $p = 0,068$; $p = 0,091$). Међутим, постојала је статистички значајна разлика у психосоцијалном статусу између испитаника у односу на степен депресије, у вези са друштвеним животом и свакодневним активностима ($p = 0,010$; $p = 0,002$).

Закључак Депресија може бити један од узрока, али и последица хроничног орофацијалног бола, а самим тим и индиректно фактор који утиче на психосоцијално стање и квалитет живота пацијената.

Кључне речи: депресија; орофацијални бол; темпоромандибуларна дисфункција

Appendix 1. Diagnostic protocol research diagnostic criteria for temporomandibular dysfunction, Dworkin & LeResche (1992)

INSTITUTION ..... PATIENT NO .....
NAME AND SURNAME ..... GENDER .....
YEAR OF BIRTH ..... OCCUPATION ..... TELEPHONE. .....

Read each question carefully and circle only one answer:

1. How would you rate your general state of health: excellent, very good, good, satisfactory or bad?
   excellent 1
   very good 2
   good. 3
   satisfactory. 4
   bad 5

2. How would you rate the condition of your oral cavity: excellent, very good, good, satisfactory or bad?
   excellent 1
   very good 2
   good. 3
   satisfactory. 4
   bad 5

3. In the last six months, have you felt pain in the area of the face, jaw, temple, in front of the ear or in the ear itself? (Axis II)
   No 0
   Yes. 1
   (if you have not felt pain in the last six months, go to question no. 14)

4a. How many years ago did you first feel such pain? (Axis II)
   ..... year
   (if the pain occurred for the first time in less than a year, skip the question and answer the following)

4b. How many months ago did you feel that pain for the first time? (Axis II) ..... months

5. Is the pain constant, occasional, or does the pain appear only once? (Axis II) Constant 1
   Occasional 2
   Only once 3

6. Have you ever sought medical help for this?
   No 1
   Yes, in the past six months 2
   Yes, more than six months ago 3

7. How would you rate your current pain on a scale of 0–10, where the value 0 corresponds to a state without pain and the value 10 to a state of unbearable pain? (Axis II)
   (no pain) (excruciating pain)
   0 1 2 3 4 5 6 7 8 9 10

8. In the last six months, on a scale of 0–10, what was your worst pain? (Axis II)
   (no pain) (excruciating pain)
   0 1 2 3 4 5 6 7 8 9 10

9. In the past six months, what is the average value of pain experienced on a scale of 0–10? (Axis II)
   (no pain) (excruciating pain)
   0 1 2 3 4 5 6 7 8 9 10

10. In the last six months, how many days did you miss work or school because of pain in the face?
   ..... days

11. In the past six months, how much did pain interfere with your daily activities, expressed on a scale of 0–10?
(no interference) (impossibility to perform activities)
0 1 2 3 4 5 6 7 8 9 10

12. In the last six months, how much have your opportunities to participate in social and family life changed due to pain, expressed on a scale of 0–10?
(no changes) (major changes)
0 1 2 3 4 5 6 7 8 9 10

13. How much did the presence of pain affect your ability to work in the last six months (including household chores), expressed on a scale of 0–10?
(no changes) (major changes)
0 1 2 3 4 5 6 7 8 9 10

14a. Has it ever happened to you that you cannot open your mouth all the way, i.e. have you had the feeling that your jaw was "locked" in some position?
No 0
Yes. 1

b. Was the limitation of mouth opening so pronounced that it prevented you from eating?
No 0
Yes. 1

15a. Do you hear a popping sound when you open or close your mouth or when you yawn?
No 0
Yes. 1

b. Do you hear a grinding noise when opening, closing or yawning?
No 0
Yes. 1

c. Have you been told or noticed that you grind your teeth or clench your jaw during sleep?
No 0
Yes. 1

d. Do you grind your teeth or clench your jaw during the day?
No 0
Yes. 1

e. Do you feel pain or have a feeling of stiffness in your jaw in the morning after waking up?
No 0
Yes. 1

f. Do you have "ringing" or any noises in your ears?
No 0
Yes. 1

Mr. Have you noticed a change in your bite when you bite down on your back teeth?
No 0
Yes. 1

16a. Have you had any other joint diseases (rheumatoid arthritis, lupus)?
No 0
Yes. 1

b. Has anyone in your family had similar joint diseases?
No 0
Yes. 1

c. Have you had or do you have swelling and pain in the area of the jaw joints?
No 0
Yes. 1

d. Does the pain you feel in the area of the jaw joints last longer than a year?
No 0
Yes. 1

17. a. Have you recently had an injury in the area of the face and jaws?
No 0
Yes. 1

b. Did you have pain before the injury?
No 0
Yes. 1

18. Have you had a headache in the past six months?
No 0
Yes. 1

19. What type of activity does the existing problem limit or prevent? (Axis II)

	No	Yes
a. chewing	0	1
b. drinking liquids	0	1
c. taking solid food	0	1
d. taking soft food	0	1
e. laughing	0	1
f. brushing teeth and washing face	0	1
g. yawning	0	1
h. swallowing	0	1
i. speech	0	1
j. facial appearance	0	1

20 a. Do you use any medications?
No 0
Yes. 1

b. How long have you been using the medication?
c. What kind of medicines do you use?
d. What dose of medicine are you using?
e. Do you take medicine regularly?
No 0
Yes. 1

Apendix 2. Symptoms check list, (SCL-90) (Axis II)

Circle only one of the offered numbers given with the offered questions.

0..... not at all
1..... very little
2..... moderately
3..... expressed
4..... exceptionally

In the past few months, how often have you been upset by:

a. headaches 0 1 2 3 4
b. loss of interest in sex or sexual pleasure 0 1 2 3 4
c. fainting or dizziness 0 1 2 3 4
d. pain in the region of the heart and chest 0 1 2 3 4
e. feeling of loss of energy or stagnation, slowness 0 1 2 3 4
f. thoughts about death or dying 0 1 2 3 4
g. loss of appetite 0 1 2 3 4
h. tearfulness 0 1 2 3 4
i. self-blame due to some events 0 1 2 3 4
j. back pain 0 1 2 3 4
k. feelings of loneliness 0 1 2 3 4
l. indifference (melancholy) 0 1 2 3 4
m. excessive worries about something 0 1 2 3 4
n. lack of interest in the environment 0 1 2 3 4
o. feeling of pain and disgust in the stomach 0 1 2 3 4
p. muscle pain 0 1 2 3 4
q. difficulty falling asleep (it takes you a long time to fall asleep) 0 1 2 3 4
r. difficulty in breathing (hard to catch your breath) 0 1 2 3 4
s. hot-cold shifts 0 1 2 3 4
t. stiffness or feeling of "numbness" in some part of the body 0 1 2 3 4
u. presence of a "lump" in the throat 0 1 2 3 4
v. feelings of hopelessness 0 1 2 3 4
w. feeling of weakness in some part of the body 0 1 2 3 4
x. feeling of heaviness in arms and legs 0 1 2 3 4
y. thoughts about ending your life 0 1 2 3 4
z. excessive intake of food 0 1 2 3 4
aa. waking up early in the morning 0 1 2 3 4
bb. restless and interrupted sleep 0 1 2 3 4
cc. feels that everything is "hard" 0 1 2 3 4
dd. feeling "caught in a clip" 0 1 2 3 4
ff. feelings of guilt 0 1 2 3 4

DOI: https://doi.org/10.2298/SARH250923082D
UDC: 616.12-008.46-07

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Albumin cobalt binding test for the diagnosis of acute coronary syndrome in patients with heart failure

Ilija Dragojević[1], Dijana Mirić[1], Bojana Kisić[1], Miroslav Milošević[1], Ljiljana Popović[1], Dragiša Rašić[1], Bratislav Bašić[2]

[1]University of Priština – Kosovska Mitrovica, Faculty of Medicine, Kosovska Mitrovica, Serbia;
[2]Institute for Student Health Care, Niš, Serbia

SUMMARY

Introduction/Objective Patients with acute coronary syndrome (ACS) and a history of heart failure (HF) constitute a distinct high-risk subgroup requiring continuous monitoring due to a greater propensity for complications. This study aimed to evaluate the diagnostic accuracy of the albumin cobalt binding (ACB) test for detecting ACS in patients with HF who presented to the hospital with acute chest pain.

Methods Patients with chest pain suggestive of ACS and either known or newly diagnosed HF were included and stratified into HF with and without ACS. Diagnostic accuracy of the ACB test was assessed statistically.

Results Among 71 patients, 26 had ACS and 45 did not. ACB test values were significantly higher in the HF with ACS group ($p < 0.0001$). The ACB test demonstrated an area under the ROC curve of 0.99 ($p < 0.0001$), with sensitivity and specificity of 96.15% and 100%, respectively. The positive predictive value was 100%, and the negative predictive value was 97.8%.

Conclusion: The ACB test appears to be a promising complementary biomarker rather than a replacement for troponin for identifying ACS in HF patients presenting with acute chest pain.

Keywords: albumin cobalt binding test; heart failure; acute coronary syndrome

INTRODUCTION

Heart failure (HF) represents a complex clinical syndrome characterized by symptoms and signs arising from structural or functional cardiac abnormalities, manifesting when the heart is unable to pump blood forward sufficiently to meet the body's metabolic needs – even when ventricular filling pressures are elevated [1].

Although acute coronary syndrome (ACS) remains a frequent precipitant of hospitalization in patients with HF, distinguishing ACS from other HF-related symptoms is challenging – clinical features and often even biomarkers (like troponins) may be inconclusive. Up to one-third of acute HF cases are precipitated by an undiagnosed ACS, highlighting the urgent need for accurate diagnostic tools to detect ACS in the context of HF. [2].

Oxidative stress plays a critical role in the development and progression of HF, particularly during ischemia–reperfusion, where excessive production of reactive oxygen species (ROS) overwhelms antioxidant systems. This redox imbalance contributes to myocardial injury through mechanisms including lipid peroxidation, oxidative modification of proteins, and DNA damage, ultimately promoting cardiomyocyte dysfunction and ventricular remodeling [3–6]. These oxidative alterations also modify the N-terminal region of serum albumin, reducing its cobalt-binding capacity, a change that can be quantified using the albumin cobalt binding (ACB) test as a surrogate for ischemia-modified albumin (IMA).

Previous studies have demonstrated elevated serum IMA levels in various cardiovascular conditions, including ACS, percutaneous coronary intervention, and HF with reduced ejection fraction [4–7]. However, there is limited evidence regarding the diagnostic utility of the ACB test specifically in HF patients with acute chest pain. Therefore, the aim of this study was to evaluate the diagnostic accuracy of the ACB test for detecting ACS in HF patients presenting with acute chest pain.

METHODS

This prospective study included patients presenting with acute chest pain suggestive of ACS who were admitted to the Coronary Care Unit of the Medical Center in Kosovska Mitrovica.

Inclusion criteria required the onset of chest pain within six hours prior to admission, and pre-established (documented in the medical record) diagnosis of HF or newly diagnosed using the Killip classification [8].

The final diagnosis of ACS was used as the reference standard. Two independent cardiologists, blinded to the ACB test results, reviewed all clinical data and assigned the diagnosis of ACS according to established guidelines [9, 10].

Received • Примљено: September 23, 2025
Accepted • Прихваћено: October 20, 2025
Online first: October 28, 2025

Correspondence to:
Ilija DRAGOJEVIĆ
University of Priština – Kosovska Mitrovica
Faculty of Medicine
Anri Dinan nn
28000 Kosovska Mitrovica
Serbia
ilija_dr@yahoo.com

Based on the presence or absence of ACS, HF patients were categorized into two groups: HF with ACS, and HF without ACS. Demographic and clinical characteristics were recorded at admission, including hypertension, diabetes mellitus, hyperlipidemia, smoking status, and previous myocardial infarction.

Upon admission, a 5 mL venous blood sample was collected and centrifuged at 3000 rpm for 15 minutes to obtain serum for routine biochemical analyses.

ACB test was conducted following the protocol described by Bar-Or et al. [11]. Briefly, patient serum was incubated with a cobalt chloride solution, followed by the addition of dithiothreitol to initiate a colorimetric reaction, which was subsequently halted using saline. The absorbance of the final mixture was measured at 470 nm using an ultraviolet-visible spectrophotometer. This approach indirectly assesses IMA levels based on its reduced binding affinity for cobalt under oxidative conditions. Within-run precision was assessed by analyzing 10 replicates of pooled serum near the optimal cut-off value, while between-day precision was determined by measuring the same sample over five consecutive days.

Data were analyzed using MedCalc version 12.3.0.0 (MedCalc Software, Ostend, Belgium). No formal sample size calculation was performed, as this was an exploratory pilot study. Measures of central tendency and variability were calculated. The Kolmogorov–Smirnov test assessed normality. For normally distributed continuous variables: results were presented as mean ± standard deviation (SD), and Student's t-test was used. For non-normally distributed variables: results were expressed as median and interquartile range (Q1-Q3), and analyzed using the Mann–Whitney U test. Categorical data were expressed as counts and percentages. The χ^2 test was applied for comparisons. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the ACB test in detecting ACS among HF patients. The optimal cut-off value was determined from the ROC curve based on the maximum Youden index: J = max (sensitivity + specificity – 1). Bootstrapped 95% confidence intervals were calculated for the Youden index and its associated cut-off.

The area under the ROC curve (AUC) with bootstrapped 95% CI was used to quantify test accuracy. Sensitivity, specificity, positive and negative predictive values, and likelihood ratios were calculated along with 95% CI. A value of $p < 0.05$ was considered statistically significant.

Ethics: The study was conducted in accordance with the Declaration of Helsinki (1975) and approved by the Ethics Committee of the Faculty of Medicine Priština – Kosovska Mitrovica (No. 05-197/9) and by the Medical Center in Kosovska Mitrovica (No. 1234). Informed consent was obtained from all participants.

RESULTS

A total of 96 patients met the initial inclusion criteria. However, 25 were excluded due to missing data, inadequate sample quality, or lack of informed consent. The final cohort consisted of 71 individuals with HF, of whom 26 were diagnosed with ACS, while 45 were not.

Baseline characteristics – including age, sex, smoking status, history of hypertension, diabetes mellitus, dyslipidemia, and previous myocardial infarction – did not significantly differ between HF patients with and without ACS (Table 1). Sex distribution was similar across all groups.

Serum ACB test results were significantly higher in HF patients with ACS compared to both the HF without ACS group. These results are summarized in Table 2. Additionally, serum albumin concentrations did not significantly differ between HF patients with and without ACS. No significant correlation was found between albumin concentrations and ACB test values ($r = –0.201$, $p = 0.22$).

Table 1. Patient characteristics

Variables	HF patients with ACS n = 26	HF patients without ACS n = 45	p
Age (years)	67 ± 9	64 ± 7	0.146
Male (%)	54%	51%	0.981
Smoking (%)	54%	49%	0.875
Hypertension (%)	70%	73%	0.923
Diabetes mellitus (%)	35%	29%	0.813
Hyperlipidemia (%)	38%	31%	0.711
Previous MI (%)	34%	13%	0.069

Data presented as mean ± standard deviation (SD) or percentage; differences between groups were calculated with t-test or χ^2 test; HF – heart failure; ACS – acute coronary syndrome; n – number of patients in group; MI – myocardial infarction

Table 2. ACB test and albumin levels

Parameter	HF with ACS (n = 26)	HF without ACS (n = 45)	p
ACB Test (ABSU)	0.86 (0.63–1.01)*	0.38 (0.28–0.42)	p < 0.0001
Albumin (g/L)	45.1 (43.0–49.6)	49.2 (46.2–51.5)	p = 0.06

Data are presented as median and interquartile range (Q1–Q3); differences between groups were calculated with Mann–Whitney test; HF – heart failure; ACS –acute coronary syndrome; n – number of participants in group; ACB test – albumin cobalt binding test; ABSU – absorbance unit

ROC curve analysis of the ACB test's diagnostic accuracy for identifying ACS among HF patients yielded an area under the ROC curve of 0.99 (95% bootstrap CI: 0.924–1.0; $p < 0.0001$) (Figure 1). The optimal diagnostic threshold was determined as 0.48 absorbance unit (95% bootstrap CI: > 0.47 to > 0.48), corresponding to a Youden index of 0.961 (95% bootstrap CI: 0.807–1.0). At this cutoff, sensitivity, specificity, positive and negative predictive values were calculated and presented in Table 3.

Analytical precision of ACB test was also confirmed. The intra-assay coefficient of variation was 4.1% at a mean value of 0.49 absorbance unit, while the inter-assay coefficient of variation was 3.6% at 0.48 absorbance unit-both measured near the established ROC cutoff.

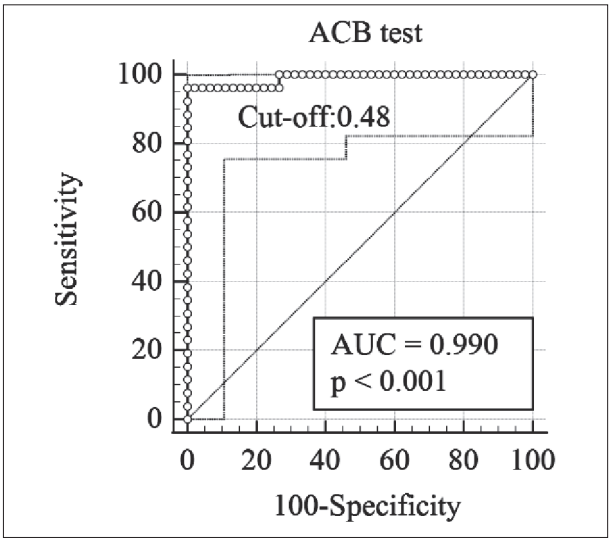


Figure 1. Receiver operating characteristic (ROC) curve of albumin cobalt binding test (ACB) test was derived for heart failure (HF) patients with acute coronary syndrome (ACS) vs. HF patients without ACS; AUC – area under the ROC curve

Table 3. Diagnostic performance of the ACB test in HF with ACS vs. HF without ACS

Parameter	Value (95% CI)
Sensitivity	96.15 (80.4–99.9)
Specificity	100 (92.1–100)
Positive predictive value	100
Negative predictive value	97.8 (86.8–99.7)
Area under ROC curve	0.99 (0.93–0.997)

Values of sensitivity, specificity, negative, and positive predictive value are presented in percentage; values in parentheses represent 95% confidence interval (CI); ACB test – albumin cobalt binding test; ACS – acute coronary syndrome; HF – heart failure

DISCUSSION

The primary finding of this exploratory study is that the ACB test shows promise for detecting ACS in patients with HF presenting with acute chest pain. In this population, where clinical symptoms may overlap and confound diagnosis, the ACB test may serve as a valuable adjunct diagnostic tool.

Patients with HF often present with symptoms indistinguishable from those of ACS, making differential diagnosis challenging. Although troponin T (cTnT) remains a cornerstone in the diagnostic assessment of ACS, its specificity is limited in the context of HF. Elevated troponin levels can occur in patients without acute ischemia, particularly in those with chronic or decompensated HF, as a result of ongoing cardiomyocyte injury and mechanical stress [12, 13, 14]. This overlap complicates clinical decision-making and underscores the need for complementary diagnostic tools capable of differentiating ischemia from other causes of biomarker elevation.

To our knowledge, this is the first study to report that the ACB test is significantly elevated in HF patients with concurrent ACS, compared to HF patients without ACS. This result supports the utility of the ACB test as a marker of myocardial ischemia [5] and left ventricular systolic dysfunction [7].

Ischemic conditions lead to oxidative alterations in the N-terminal region of human serum albumin, resulting in reduced binding affinity for metal ions such as cobalt. These structural changes are primarily attributed to increased ROS activity during ischemia and reperfusion, which modify amino acid residues essential for metal coordination. The ACB test exploits this biochemical shift as an indirect indicator of myocardial ischemia, reflecting oxidative stress-induced albumin dysfunction [5, 15, 16].

There is a growing body of evidence implicating oxidative stress in the pathogenesis of various cardiovascular conditions, including myocardial ischemia-reperfusion injury, atherosclerosis, cardiac hypertrophy, hypertension, diabetic cardiomyopathy, and atrial fibrillation [17]. The transition from myocardial hypertrophy to HF is associated with progressive oxidative damage, highlighting the role of ROS in disease progression [18]. While the etiological background of HF is heterogeneous, factors such as coronary artery disease, diabetes, and hypertension are major contributors to the observed oxidative alterations [19]. Notably, oxidative stress is elevated in both ischemic and non-ischemic HF [20]. During ischemia-reperfusion, the excessive generation of oxygen radicals can overwhelm antioxidant defenses, resulting in significant tissue damage [21]. These changes may further impair the albumin-metal binding capacity, which is the basis of the ACB test.

Patients with HF and concomitant ACS represent a high-risk subgroup with greater rates of complications, prolonged hospital stays, and increased mortality during hospitalization and at follow-up intervals of 30 days and one year [2, 22]. Early identification of ACS in these patients is thus of paramount clinical importance.

In our study, the ACB test demonstrated high diagnostic performance: sensitivity and specificity were 96.2% and 100%, respectively, with a positive predictive value of 100% and a negative predictive value of 97.8%. However, the very high AUC of 0.99 is unlikely to be replicated in larger, more heterogeneous populations. These findings suggest that the ACB test could facilitate early identification of ACS in HF patients, potentially enabling faster treatment and more efficient triage in emergency settings.

Moreover, previous studies have indicated that patients with HF undergo fewer coronary angiographies and revascularization procedures. However, when complete revascularization is achieved, outcomes are significantly improved, with reductions in HF hospitalizations and cardiovascular mortality [23]. Rapid and reliable differentiation of ACS from HF-related symptoms is essential to enable appropriate clinical interventions, such as early revascularization. Incorporating the ACB test into early diagnostic workflows could help identify high-risk patients who would benefit from timely coronary angiography and targeted therapy, ultimately improving outcomes through prompt ischemia management [22, 23].

It should be noted that this cohort was enrolled in 2014, when a third-generation cTnT assay was in use. Although high-sensitivity cardiac troponins (hs-cTn) are now the diagnostic standard, their interpretation in HF remains

problematic because chronically elevated baseline levels can mask acute ischemia. In this setting, the ACB test-capturing ischemia-driven albumin modification – could serve as a complementary marker. Our results suggest that ACB may be most useful in patients falling into the "observe" zone of accelerated 0/1-h or 0/2-h hs-cTn algorithms, and in HF patients with persistently elevated hs-cTn where additional ischemic evidence is needed [24, 25, 26]. Comparable evidence from a Serbian chest-pain cohort demonstrated that the HEART score achieved an AUROC of 0.771 (sensitivity 89.2%, specificity 57.6%) for detecting ischemic heart disease, underscoring the need for adjunct biomarkers such as ACB to improve early ACS detection in high-risk patients [27].

Taken together, the time-stable pathophysiology captured by ACB, the persistent interpretive limitations of hs-cTn in HF, and evidence that accelerated algorithms may underperform in certain high-risk patients support the continuing relevance of our results. Rather than proposing ACB as a replacement, we highlight its potential role as a complementary ischemia marker that deserves prospective validation within guideline-endorsed 0/1-h or 0/2-h hs-cTn frameworks [7, 8, 25, 26].

The limitations of this study must be acknowledged. Small sample size and single-center design reduce generalizability. Restriction to patients within six hours of symptom onset limits applicability to later presenters. No head-to-head ROC analysis of cTnT *vs*. ACB prevents direct comparative conclusions. Near-perfect ROC performance may reflect optimism bias.

CONCLUSION

This study suggests that the albumin ACB test is a promising complementary biomarker for detecting ACS in HF patients presenting with acute chest pain, based on data collected in 2014 with third-generation troponin assays. The test exhibited high sensitivity, specificity, and predictive values. However, these findings should be interpreted as preliminary, pilot-level evidence requiring validation in larger multicenter studies embedded within guideline-recommended hs-cTn diagnostic algorithms.

Conflict of interest: None declared.

REFERENCES

1. Bozkurt B, Coats AJS, Tsutsui H, Abdelhamid CM, Adamopoulos S, Albert N, et al. Universal definition and classification of heart failure: a report of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology, Japanese Heart Failure Society and Writing Committee of the Universal Definition of Heart Failure: Endorsed by the Canadian Heart Failure Society, Heart Failure Association of India, Cardiac Society of Australia and New Zealand, and Chinese Heart Failure Association. Eur J Heart Fail. 2021;23(3):352–80. [DOI: 10.1002/ejhf.2115] [PMID: 33605000]
2. Harjola VP, Parissis J, Bauersachs J, Brunner-La Rocca HP, Bueno H, Čelutkienė J, et al. Acute coronary syndromes and acute heart failure: a diagnostic dilemma and high-risk combination. A statement from the Acute Heart Failure Committee of the Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail. 2020;22(8):1298–314. [DOI: 10.1002/ejhf.1831] [PMID: 32347648]
3. Valaitienė J, Laučytė-Cibulskienė A. Oxidative stress and its biomarkers in cardiovascular diseases. Artery Res. 2024;30(1):18. [DOI: 10.1007/s44200-024-00062-8]
4. Zhao C, Liu T, Wei H, Li J. Serum oxidative stress factors predict myocardial ischemia reperfusion injury after percutaneous coronary intervention in patients with acute myocardial infarction and type 2 diabetes mellitus. Postepy Kardiol Interwencyjnej. 2023;19(4):333–42. [DOI: 10.5114/aic.2023.133475] [PMID: 38187486]
5. Ralapanawa U, Sivakanesan R, Tennakoon S, Karunathilake P. Ischemia-modified albumin: is it a promising marker in acute coronary syndrome? BMC Cardiovasc Disord. 2024;24(1):436. [DOI: 10.1186/s12872-024-04108-2] [PMID: 39174920]
6. Shin H, Kim JG, Jang BH, Lim TH, Kim W, Cho Y, et al. Diagnostic accuracy of ischemia-modified albumin for acute coronary syndrome: a systematic review and meta-analysis. Medicina (Kaunas). 2022;58(5):614. [DOI: 10.3390/medicina58050614] [PMID: 35630031]
7. Shevtsova A, Gordiienko I, Tkachenko V, Ushakova G. Ischemia-Modified Albumin: Origins and Clinical Implications. Dis Markers. 2021;2021:9945424. [DOI: 10.1155/2021/9945424] [PMID: 34336009]
8. Bassand JP, Hamm CW, Ardissino D, Boersma E, Budaj A, Fernández-Avilés F, et al. Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. Eur Heart J. 2007;28(13):1598–660. [DOI: 10.1093/eurheartj/ehm161] [PMID: 17569677]
9. Van de Werf F, Bax J, Betriu A, Blomstrom-Lundqvist C, Crea F, Falk V, et al. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the management of ST-segment elevation acute myocardial infarction of the ESC. Eur Heart J. 2008;29(23):2909–45. [DOI: 10.1093/eurheartj/ehn416] [PMID: 19004841]
10. Thygesen K, Alpert JS, White HD, Jaffe AS, Apple FS, Galvani M, et al. Universal definition of myocardial infarction. Eur Heart J. 2007;28(20):2525–38. [DOI: 10.1093/eurheartj/ehm355] [PMID: 17951287]
11. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia – a preliminary report. J Emerg Med. 2000;19(4):311–5. [DOI: 10.1016/s0736-4679(00)00255-9] [PMID: 11074321]
12. Kubo T, Ochi Y, Baba Y, Sugiura K, Takahashi A, Hirota T, et al. Elevation of high-sensitivity cardiac troponin T and left ventricular remodelling in hypertrophic cardiomyopathy. ESC Heart Fail. 2020;7(6):3593–600. [DOI: 10.1002/ehf2.12852] [PMID: 33047518]
13. Kim BS, Kwon CH, Chang H, Choi JH, Kim HJ, Kim SH. The association of cardiac troponin and cardiovascular events in patients with concomitant heart failure preserved ejection fraction and atrial fibrillation. BMC Cardiovasc Disord. 2023;23(1):273. [DOI: 10.1186/s12872-023-03302-y] [PMID: 37226163]
14. Hu H, Li J, Wei X, Zhang J, Wang J. Elevated level of high-sensitivity cardiac troponin I as a predictor of adverse cardiovascular events in patients with heart failure with preserved ejection fraction. Chin Med J (Engl). 2023;136(18):2195–202. [DOI: 10.1097/CM9.0000000000002639] [PMID: 37279378]
15. Gerdan Z, Saylan Y, Denizli A. Biosensing Platforms for Cardiac Biomarker Detection. ACS Omega. 2024;9(9):9946–60. [DOI: 10.1021/acsomega.3c06571] [PMID: 38463295]
16. Zoroddu S, Zinellu A, Carru C, Sotgia S. Analytical insights into methods for measuring ischemia-modified albumin. Molecules. 2024;29(19):4636. [DOI: 10.3390/molecules29194636] [PMID: 39407566]
17. Jin S, Li H, Chen Y, Wang J, Zhao P, Zhang Y, et al. Advances in management of oxidative stress in cardiovascular diseases: a systematic review. Antioxidants (Basel). 2024;13(8):923. [DOI: 10.3390/antiox13080923] [PMID: 39199169]
18. Guo S, Hu Y, Ling L, Yang Z, Wan L, Yang X, et al. Molecular mechanisms and intervention approaches of heart failure (Review). Int J Mol Med. 2025;56(2):125. [DOI: 10.3892/ijmm.2025.5566] [PMID: 40511535]

19. Kong AS, Lai KS, Hee CW, Loh JY, Lim SHE, Sathiya M. Oxidative Stress Parameters as Biomarkers of Cardiovascular Disease towards the Development and Progression. Antioxidants (Basel) 2022;11(6):1175. [DOI: 10.3390/antiox11061175] [PMID: 35740071]
20. D'Amato A, Cestiè C, Ferranti F, Segato C, Prosperi S, Germanò R, et al. Implications of oxidative stress in the pathophysiological pathways of heart failure. Int J Mol Sci. 2025;26(11):5165. [DOI: 10.3390/ijms26115165] [PMID: 40507974]
21. Duan D, Fan T, Zhang L, Li L, Wang H, Guo M, et al. The Correlation Between Cardiac Oxidative Stress and Inflammatory Cytokine Response Following Myocardial Infarction. Clin Appl Thromb Hemost. 2023;29:10760296231211907. [DOI: 10.1177/10760296231211907] [PMID: 37933137]
22. Goodwin NP, Clare RM, Harrington JL, Badjatiya A, Wojdyla DM, Udell JA, et al. Morbidity and Mortality Associated With Heart Failure in Acute Coronary Syndrome: A Pooled Analysis of 4 Clinical Trials. J Card Fail. 2023;29(12):1603–14. [DOI: 10.1016/j.cardfail.2023.07.004] [PMID: 37479054]
23. Bruno F, Marengo G, De Filippo O, Wanha W, Leonardi S, Raposeiras Roubin S, et al. Impact of complete revascularization on development of heart failure in patients with acute coronary syndrome and multivessel disease: a subanalysis of the CORALYS registry. J Am Heart Assoc. 2023;12(15):e028475. [DOI: 10.1161/JAHA.122.028475] [PMID: 37489724]
24. Byrne RA, Rossello X, Coughlan JJ, Barbato E, Berry C, Chieffo A, et al. 2023 ESC Guidelines for the management of acute coronary syndromes. Eur Heart J. 2023;44(38):3720–826. [DOI: 10.1093/eurheartj/ehad191] [PMID: 37622654]
25. Sandoval Y, Apple FS, Mahler SA, Body R, Collinson PO, Jaffe AS; International Federation of Clinical Chemistry and Laboratory Medicine Committee on the Clinical Application of Cardiac Biomarkers. High-Sensitivity Cardiac Troponin and the 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR Guidelines for the Evaluation and Diagnosis of Acute Chest Pain. Circulation. 2022;146(7):569–81. [DOI: 10.1161/CIRCULATIONAHA.122.059678] [PMID: 35775423]
26. Ashburn NP, Snavely AC, O'Neill JC, Allen BR, Christenson RH, Madsen T, et al. Performance of the European Society of Cardiology 0/1-Hour Algorithm With High-Sensitivity Cardiac Troponin T Among Patients With Known Coronary Artery Disease. JAMA Cardiol. 2023;8(4):347–56. [DOI: 10.1001/jamacardio.2023.0031] [PMID: 36857071]
27. Stojković T, Stojković E, Sakač D, Redžek A, Stojšić-Milosavljević A, Velicki L, et al. Role of HEART score in prediction of coronary artery disease and major adverse cardiac events in patients presenting with chest pain. Srp Arh Celok Lek. 2022;150(3–4):149–55. [DOI: 10.2298/SARH220213038S]

Тест везивања албумина за кобалт у дијагностици акутног коронарног синдрома код болесника са срчаном инсуфицијенцијом

Илија Драгојевић[1], Дијана Мирић[1], Бојана Кисић[1], Мирослав Милошевић[1], Љиљана Поповић[1], Драгиша Рашић[1], Братислав Башић[2]

[1]Универзитет у Приштини – Косовска Митровица, Медицински факултет, Косовска Митровица, Србија;
[2]Завод за здравствену заштиту студената, Ниш, Србија

САЖЕТАК

Увод/Циљ Болесници са акутним коронарним синдромом (АКС) и историјом срчане инсуфицијенције (СИ) чине посебну подгрупу високог ризика која захтева континуирано праћење због веће склоности ка компликацијама.
Циљ ове студије био је да се процени дијагностичка тачност теста везивања кобалта за албумин (*ACB* тест) у откривању АКС код болесника са СИ који су хоспитализовани са акутним болом у грудима.

Методе У истраживање су укључени болесници са болом у грудима који је указивао на АКС, а који су имали познату или новодијагностиковану СИ. Болесници су стратификовани у групе са СИ и АКС и са СИ без АКС. Дијагностичка тачност *ACB* теста процењена је статистичком анализом.

Резултати Од укупно 71 болесника, 26 је имало АКС, док 45 није. Вредности *ACB* теста биле су значајно више у групи са СИ и АКС ($p < 0,0001$). *ACB* тест је показао површину испод *ROC* криве од 0,99 ($p < 0,0001$), са сензитивношћу и специфичношћу од 96,15% и 100%, редом. Позитивна предиктивна вредност износила је 100%, а негативна 97,8%.

Закључак Резултати указују да *ACB* тест може имати улогу комплементарног биомаркера у препознавању АКС код болесника са СИ, али да не може заменити тропонин.

Кључне речи: тест везивања албумина за кобалт; срчана инсуфицијенција; акутни коронарни синдром

DOI: https://doi.org/10.2298/SARH250627086D
UDC: 616.832-004

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Immunology of bone marrow CD34 subsets and clonal hematopoiesis of indeterminate potential in amyotrophic lateral sclerosis

Igor S. Dolgopolov[1], Ludmila Y. Grivtsova[2], Nikolai I. Kovalenko[1], Petr A. Shatalov[3], Andrey S. Bryukhovetsky[1], Maxim Yu. Rykov[4,5]

[1]JSC Neurovita Clinical Hospital, Moscow, Russia;
[2]A. Tsyb Medical Radiological Research Center, Kaluga Region, Obninsk, Russia;
[3]National Medical Research Radiological Center, Department of Molecular Genetic Service, Moscow, Russia;
[4]Russian State Social University, Moscow, Russia;
[5]Central Scientific Research Institute of Healthcare Organization and Informatization, Moscow, Russia

SUMMARY

Introduction Recent advances in pathogenesis of neurodegenerative diseases have shown that inflammation is a key factor of progression.

Methods The amyotrophic lateral sclerosis (ALS) group included 10 patients [mean age 55.1 ± 3.1 years (CI 95%: 48–62.2)]. Whole-exome sequencing and immunophenotyping of CD34+ subsets in bone marrow (BM) cells were performed before the start of therapy (baseline) and six months later (follow-up). The control group included 10 healthy donors, mean age was 39.9 ± 3.9 years (CI 95%: 31.2–48.6).

Results The peripheral blood stem cells (PBSCs) were collected after four-day granulocyte colony-stimulating factor administration. The total mean number of collected CD34+ cells was $290.4 \pm 53.5 \times 10^6$ (CI 95%: 177.6–403.3). Patients received fludarabine 25 mg/m^2/day, on days one and two. To induce hematopoietic stem cell transdifferentiation the PBSCs were incubated with human placenta double-stranded DNA fragments *ex vivo* and reinfused 48 hours post fludarabine. Clonal hematopoiesis of indeterminate potential (CHIP) was detected in three cases (30%) before therapy. A significant increase CD34+CD13+ and CD34+CD123+ hematopoietic stem cells (HSCs) in BM was detected. The CD34+CD44+ level significantly decreased. Levels of CD34+CD7+, CD34+CD2+ and CD34+CD56+ showed a trend toward increased mean value after treatment. In two cases CHIP disappeared, in one case a decrease in the allelic variant frequency has been shown. The mean amyotrophic lateral sclerosis functional rating scale-revised score did not change [39 ± 0.6 points (CI 95%: 37.6–40.4) *vs*. 40.4 ± 0.7 (CI 95%: 38.8–42)].

Conclusion Our study is the first attempt to characterize the subsets of BM HSCs in ALS. They demonstrate that BM is able to respond to immune-mediated neuroinflammation. Preliminary results indicate a possible link between CHIP and ALS and point the way to eliminating aberrant clones.

Keywords: CD34 subsets; bone marrow; amyotrophic lateral sclerosis; clonal hematopoiesis of indeterminate potential; fludarabine

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting primarily the motor system leading to paralysis and death within 3–5 years after the onset of symptoms. Most cases of classic ALS (~90%) are identified as sporadic, with little genetic contribution [1]. The identification of *C9orf72* mutations in patients without a family history and disease discordance in monozygotic twins challenge the traditional binary classification of the disease into familial and sporadic forms and suggest a strong contribution of epigenetic factors in ALS [2]. Sporadic ALS is clinically indistinguishable from familial forms, raising the possibility that the disease is caused by the interaction of several genes and/or epigenetic dysregulation of their function. Evidence is provided by the fact that the major epigenetic differences arise in lymphocytes, skeletal muscle and fat during the lifetime of monozygotic twins [3]. Somatic mosaicism, that is, the presence of several genetically different cell clones in the same tissue, is an inevitable consequence of human aging [4].

Somatic mutations also occur in hematopoietic stem cells (HSCs), the offsprings of which participate in immunopoiesis and give rise to some neuroglial cells. The expanded blood cell clones with mutations in driver genes and/or genetic alterations in chromosomes have been identified. This phenomenon is particularly prevalent in the elderly and the greatest known risk factor for ALS is aging [2, 4, 5, 6]. Some recent works have attempted to trace the connection between ALS and clonal hematopoiesis of indeterminate potential (CHIP) [7].

Recent advances in pathogenesis of neurodegenerative diseases have shown that inflammation is not only a result of neurodegeneration, but also a key factor in this process. Protein aggregates, which are a very common pathologic phenomenon in neurodegeneration, are now perceived rather as a consequence of the immune system's dysfunction in maintaining

Received • Примљено: June 27, 2025
Revised • Ревизија: November 14, 2025
Accepted • Прихваћено: November 19, 2025
Online first: November 25, 2025

Correspondence to:
Igor S. DOLGOPOLOV
JSC Neurovita Clinical Hospital
Marshal Timoshenko 7c1
121359 Moscow
Russia
irdolg@rambler.ru

the genetic homeostasis of the body then as the cause of the disease. Increasing evidence suggests that common risk factors for neurodegenerative diseases may trigger an inflammatory response, initiating and exacerbating disease progression. In addition, many genetic risk factors for neurodegenerative diseases are associated with immunity. The interleukin (IL) family, especially IL-1, IL-6 and IL-17, plays a critical role in the pathogenesis of these diseases. It has been shown that patients with ALS have a significant decrease in Treg and FoxP3 protein expression. [7]. Moreover, the CD3, CD4, CD8 and CD3+CD56+ T cells, natural killer (NK) cells, monocytes and neutrophils were found to be increased in ALS patients and also associated with disease progression. While higher levels of effector CD4 T cells in both blood and cerebrospinal fluid (CSF) were associated with decreased survival, an increased frequency of activated regulatory T cells (Treg) in blood was associated with improved survival [8].

It can be assumed that clonal disorders of hematopoiesis at the level of a bone marrow (BM) stem cell that has acquired a somatic mutation or chromosomal aberration play an important role in the pathogenesis of neurodegenerative diseases, and ALS in particular. The progeny of such as HSCs acquires a clonal advantage, leading to their clonal expansion, development of chronic immune-mediated inflammation and disruption of the innate immune system. The degree of nervous system damage, the variety of clinical manifestations, the time of disease onset, and some other phenomena may vary depending on the repertoire of tissue somatic/germline mutations as well as the genes of HSCs involved in the somatic mutation, their differentiation, secreted cytokines and activated signaling pathways. The role of BM hematopoietic cells in the development and maintenance of immune-mediated inflammation and demyelination was recently demonstrated in patients with multiple sclerosis (MS) [9].

Planning this study, we hypothesized that since BM is the central organ of hematopoiesis (both lympho- and myelopoiesis), it may be possible to restart and correct the abnormal immune response in ALS using minimal immunomodulation and *ex vivo* transdifferentiated autologous HSCs.

METHODS

Patients

The ALS group included 10 patients with confirmed disease (four men, six women), with a mean disease duration from the time of diagnosis 26.5 ± 4 months (CI 95%: 17.5–35.6). The median age was 53.9 ± 9.9 years (CI 95%: 28–47). The median ALS functional rating scale-revised (ALSFRS-R) score was 39 ± 0.6 points (CI 95%: 37.6–40.4). The median Karnofsky score at the time of inclusion in the study was 53 ± 3 (CI 95%: 46–60). All patients underwent BM puncture prior to therapy start in protocol. Whole-exome sequencing and immunophenotyping of CD34+ subsets in BM cells were performed at the baseline in "a steady state" before the administration of granulocyte colony-stimulating factor (G-CSF) (baseline) and during the first six months of follow-up, but not before three months (follow-up). The control group included 10 BM donors (seven men, three women), the median age of the donors was 39.9 ± 3.9 years (CI 95%: 31.2–48.6, p = 0.07). Toxicity of therapy was assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (NCI CTCAE v5.0 scale).

Targeted gene panel sequencing

Targeted paired-end sequencing was performed on an MGISEQ-G400 instrument (MGI Tech Co. Ltd., Shenzhen, China) utilizing either Roche KAPA HyperExome (Roche Holding AG, Basel, Switzerland) or Nanodigmbio NEXome Plus Panel v1.0 (Nanodigmbio Pte. Ltd., Nanjing, China) whole-exome panels to a median sequencing depth of ~100 × across sites. For the germline and somatic analyses pipeline, the following software tools were employed: BWA2 v2.2.1 (Intel Corp., Santa Clara, CA, USA) for alignment to the human reference genome (*GRch37*), Streammd v4.3.0 (QIMR Berghofer Medical Research Institute, Herston, Australia) for marking and removal of polymerase chain reaction (PCR) duplicates, Sambamba v1.0.1 (Free Software Foundation, Inc., Boston, MA, USA) for filtering mapped variants.

For the germline analysis pipeline, the following software tools were employed: BWA2 v2.2.1 (Intel Corp.) for alignment to the human reference genome (*GRch37*), Streammd v4.3.0 (QIMR Berghofer Medical Research Institute) for marking and removal of PCR duplicates, Sambamba v1.0.1 (Free Software Foundation, Inc.) for filtering mapped variants, Genome Analysis Toolkit (GATK) Picard for quality control and mapping metrics assessment, Google Deepvariant v1.6.1 for nucleotide substitution detection, Bcftools v1.20 (Free Software Foundation, Inc.) for variant filtering, and Ensembl Variant Effect Predictor release 112 (Ensembl, Cambridge, UK) for variant annotation.

The somatic analysis pipeline utilized several shared tools including BWA2 v2.2.1 (Intel Corp.) for genome alignment, Streammd v4.3.0 (QIMR Berghofer Medical Research Institute) for PCR duplicate processing, and Sambamba v1.0.1 (Free Software Foundation, Inc.) for variant filtering. However, it diverged in using GATK v4.4.0.0 for quality control and mapping metrics, GATK Mutect 2 for nucleotide substitution identification, and FINGS v1.7.2 for variant filtering, while maintaining Ensembl Variant Effect Predictor release 112 (Ensembl) for annotation.

The analysis focused on a panel of genes implicated in relevant pathways, including *ASXL1*, *ASXL2*, *BRCC3*, *CBL*, *DNMT3A*, *ETNK1*, *GNAS*, *GNB1*, *IDH1*, *IDH2*, *JAK2*, *KRAS*, *NRAS*, *PPM1D*, *SETBP1*, *SF3B1*, *SRSF2*, *TET2*, *TP53*, *U2AF1*, *PTPN11*, *BCOR*, *BCORL1*, *UBA1*, *CTCF*, *DNMT1*, *DNMT3b*, *AKT1*, *MYD88*, *NLRP3*, *MTOR*, *NANOG*, *OCT4*, *PTEN*, and *SOX2*.

BM immunophenotyping by flow cytometry

The analysis of CD34 subset was performed using multicolor 5–6-parameter flow cytometry on a FACS CANTO II flow cytometer (BD, Franklin Lakes, NJ, USA) as described previously [10]. From 1,000,000 to 2,000,000 events (all cells in the sample) were recorded from each sample during flow cytometric analysis. Flow cytometry data were evaluated using Kaluza Analysis software, version 3.1 (Beckman Coulter, Brea, CA, USA). Pluripotent cell populations were characterized based on the combined expression on CD34+ cells of the antigens CD45, CD44, CD33, CD13, CD41, CD123, CD133, CD117, CD61, CD10, CD19, CD2, CD7 and CD56.

The gating sequence for CD34 subpopulation analysis was as follows: nucleated cells were identified based on SYTO16 expression in the side-angle scatter (SSC) *vs*. SYTO16 parameters. Within SYTO-positive cells, a gate was determined for CD34+ cells in the SSC *vs*. CD34 parameters. Next, expression of the antigens of interest was assessed within nucleated SYTO+CD34+ cells. Internal positive controls served as positivity controls. Each population was assessed separately in the parameters – target antigen (ordinate axis) *vs*. CD34 (abscissa axis).

Data analysis

Data on CDs were analyzed using the inverse variance of the as the mean ± SD, 95% confidence intervals (CI) and p values. The Wilcoxon signed-rank for ALS baseline *vs*. follow-up and t-test for donors *vs*. ALS baseline were used. Significance was set at $p \leq 0.05$ and a CI 95%. The data in the figures are presented as medians and degrees of dispersion. Student's t-test was used to compare continuous variables. Statistical analyses were based on a database snapshot taken on February 28, 2025, and performed using SPSS for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). The primary endpoint was the change in the expression of the surface molecules in the CD34+ cell subpopulation between baseline and follow-up. Secondary endpoints included feasibility, changes in the CHIP allelic variant frequency (VAF) (if available) and the ALSFRS-R score.

Ethics: All patients signed informed consent to participate in the study. The protocol was approved by Ethics Committee at Far Eastern Federal University (№204-2023).

RESULTS

The patients received granulocyte colony-stimulating factor (5 μg/kg/day) subcutaneously for four days. The peripheral blood mononuclear cells (PBMCs) were collected on a Spectra Optia continuous flow separator (Terumo BCT Inc., Lakewood, CO, USA). Patients received fludarabine 25 mg/m^2/day, on days one and two (SD = 50 mg/m^2). The dose of fludarabine was selected to ensure lymphodepletion and effects on memory T cells while minimizing side effects. Previously harvested CD34+ cells were reinfused intravenously 48 hours after immunomodulatory therapy after *ex vivo*. incubation with human placenta double-stranded DNA fragments (Panagen®, Panagen LLC, Millersville, PA, USA) to induce HSC proliferation. Details of the clinical study protocol of Panagen® and its mechanism of action on a hematopoietic stem cell can be found in [11].

Two patients (20%) experienced transient increasing sensation of weakness and difficulty breathing during filgrastim administration. None of the patients experienced > grade 1 NCI CTCAE v5.0 toxicity during fludarabine therapy. The mean reinfusion dose was $2 \pm 0.3 \times 10^6$ CD34+/kg (CI 95%: 1.3–2.6). Median follow-up from the start of the therapy to control workup was 5 ± 1 months (CI 95%: 4.6–6). The mean ALSFRS-R score at the time of control workup was 40.4 ± 0.7 (CI 95%: 38.8–42), $p > 0.05$.

Somatic mutations were detected in three patients (30%) before therapy. After therapy, somatic mutations were not detected in two patients. A decrease in VAF was observed in one case (Table 1). No somatic mutations were detected in the donors.

No significant differences were found between CD34-subsets in healthy donors and ALS patients at baseline examination (Table 2).

Despite the fact that the number of CD34+ HSCs in the BM of ALS patients before and after therapy was not

Table 1. Dynamics of somatic mutations in patients with amyotrophic lateral sclerosis during therapy

Patients	Age (years)	Somatic mutation in bone marrow (gene, dbSNP, VAF (%))	
		Before therapy (baseline)	After therapy (follow-up)
#1	66.6	*DNMT3A*, rs151168784, 6.6%	*DNMT3A*, rs151168784, 3.9%
#2	45.5	*PTEN*, rs35632884, 6.4%	Not detected
#3	54.6	*ASXL1*, rs2011586997, 3% *ASXL1*, rs2145387839, 2.4% *CBL*, rs886041500, 3%	Not detected

dbSNP – database of single nucleotide polymorphisms; VAF – variant allele frequency

Table 2. Subsets of CD34+ cells in bone marrow of amyotrophic lateral sclerosis (ALS) patients before treatment compared to healthy donors

Subsets	Healthy donors, % (mean ± SD)	ALS, baseline, % (mean ± SD)	p
CD34+	0.9 ± 0.09	0.78 ± 0.11	> 0.05
CD34+CD38+	81.9 ± 4.4	67.6 ± 6.7	
CD34+CD13+	71.8 ± 4.2	63.7 ± 3.7	
CD34+CD33+	48.1 ± 8.6	47.7 ± 7.7	
CD34+CD2+	5.9 ± 0.9	5.2 ± 1.3	
CD34+CD7+	2.7 ± 1.1	3.2 ± 1.2	
CD34+CD10+	21.3 ± 3	10.9 ± 3.9	
CD34+CD19+	17.6 ± 3.3	13.5 ± 4.1	
CD34+CD90+	29.5 ± 4.7	28.0 ± 5.7	
CD34+CD56+	2.8 ± 1.8	3.3 ± 1.1	
CD34+CD123+	22.9 ± 8.6	6.4 ± 0.95	
CD34+CD133+	66.0 ± 3	60.2 ± 5.2	
CD34+CD41+	6.4 ± 0.7	5.6 ± 1.7	
CD34+CD44+	52.9 ± 9.2	62.6 ± 7.6	
CD34+CD61+	5.5 ± 1.5	4.3 ± 1	
CD34+CD117+	76.6 ± 1.8	80.0 ± 3.2	
CD34+HLA-DR	90.5 ± 3.7	81.7 ± 3	
CD34+CD45low	88.6 ± 2.3	91.4 ± 1.6	

SD – standard deviation; HLA – human leukocyte antigen

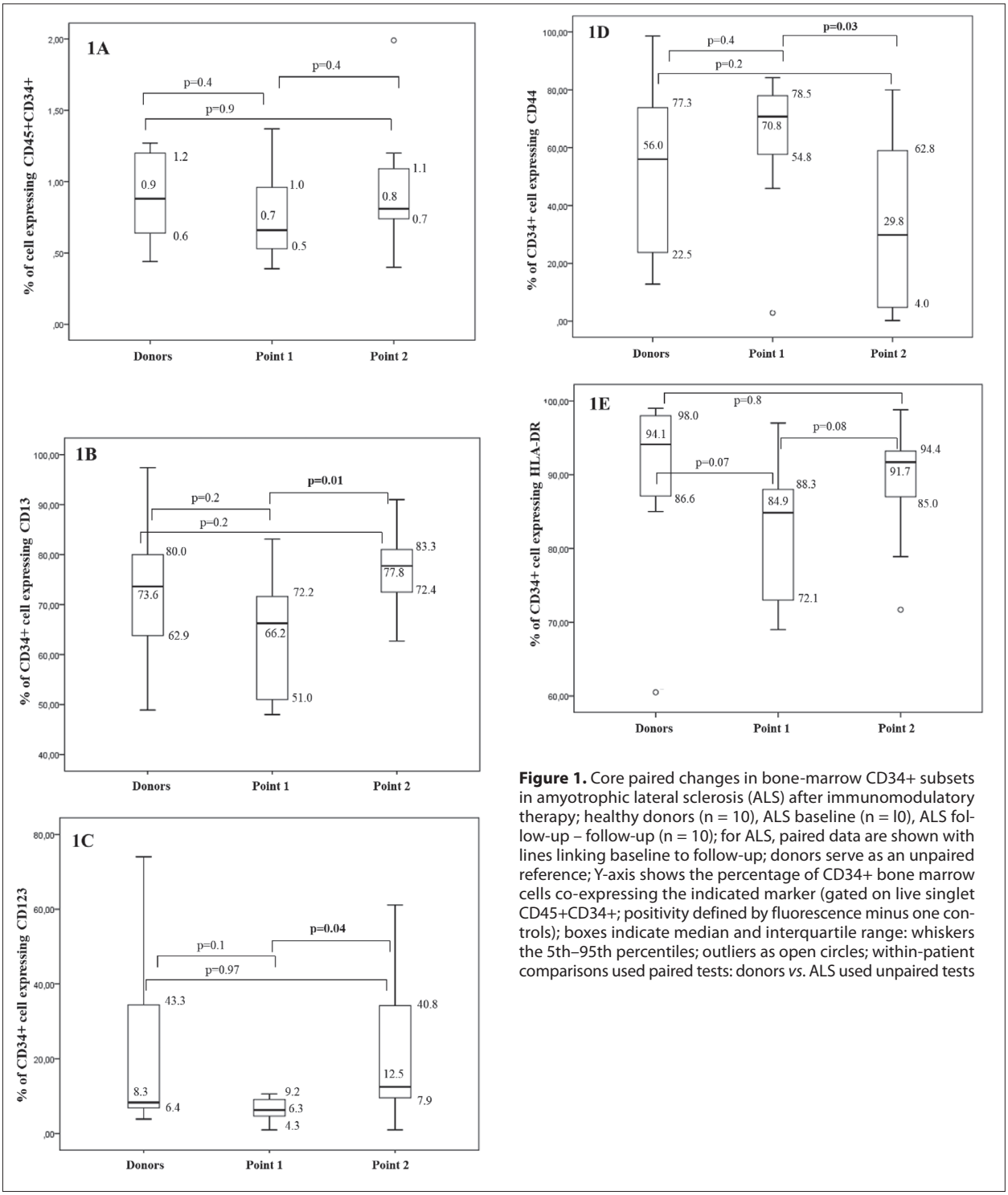


Figure 1. Core paired changes in bone-marrow CD34+ subsets in amyotrophic lateral sclerosis (ALS) after immunomodulatory therapy; healthy donors (n = 10), ALS baseline (n = l0), ALS follow-up – follow-up (n = 10); for ALS, paired data are shown with lines linking baseline to follow-up; donors serve as an unpaired reference; Y-axis shows the percentage of CD34+ bone marrow cells co-expressing the indicated marker (gated on live singlet CD45+CD34+; positivity defined by fluorescence minus one controls); boxes indicate median and interquartile range: whiskers the 5th–95th percentiles; outliers as open circles; within-patient comparisons used paired tests: donors *vs*. ALS used unpaired tests

significantly different (0.9% ± 0.09% (CI 95%: 0.7–1.1) and 0.78% ± 0.11% (CI 95%: 0.53–1), respectively), the level of CD34+ cells after therapy (follow-up) approached the values of healthy donors (Figure 1A). A significant increase in the number of CD34+CD13+ and CD34+CD123+ HSCs was detected after the reinfusion of transdifferentiated CD34+ HSCs [63.7 ± 0.9 (CI 95%: 55.3–72) *vs.* 78.1 ± 2.6 (CI 95%: 72.2–84.1) and 6.4 ± 0.9 (CI 95%: 4.2–8.5) *vs.* 23.4 ± 7.1 (CI 95%: 7.4–39.4) at baseline and follow-up, respectively]. The level of CD34+CD44+ in BM significantly decreased from [62.6 ± 7.6 (CI 95%: 45.4–79.7) to 34 ± 9.5 (CI 95%: 12.4–55.5) at baseline and follow-up, respectively]. There was a trend towards a decrease in surface expression level of class II (human leukocyte antigen) molecules on CD34+ in ALS patients before the start of therapy with its subsequent recovery [81.7 ± 3 (CI 95%: 75.0–88.4) *vs.* 89.5 ± 8.4 (CI 95%: 83.5–95.5) at baseline and follow-up, respectively] (Figure 1B–1E). Levels of lymphoid-leaning CD34+CD2+ and CD34+CD56+, but not CD34+CD7+, showed a trend toward increased mean value and widened confidence intervals compared with the values before treatment (baseline), although they did not reach statistical

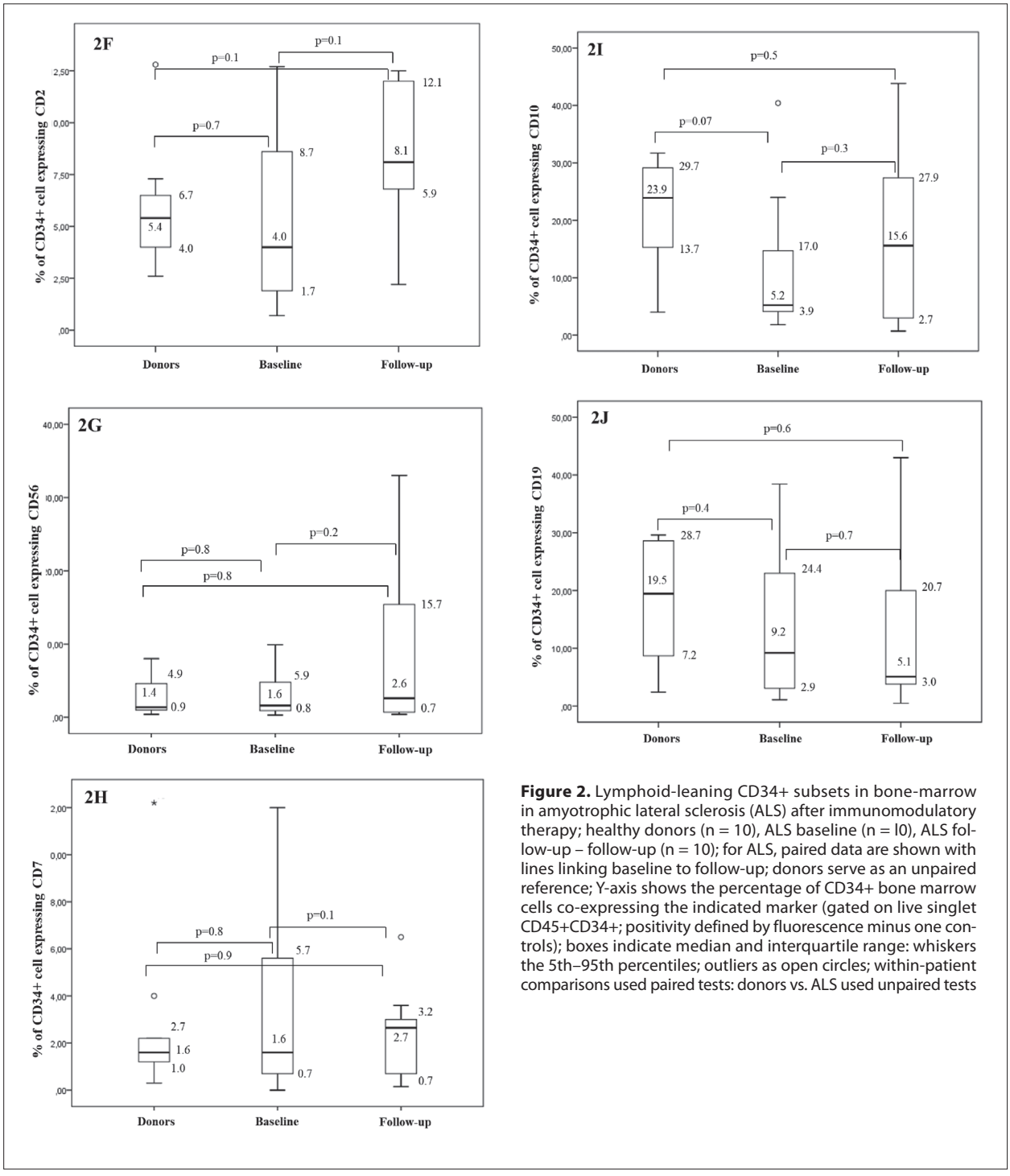


Figure 2. Lymphoid-leaning CD34+ subsets in bone-marrow in amyotrophic lateral sclerosis (ALS) after immunomodulatory therapy; healthy donors (n = 10), ALS baseline (n = l0), ALS follow-up – follow-up (n = 10); for ALS, paired data are shown with lines linking baseline to follow-up; donors serve as an unpaired reference; Y-axis shows the percentage of CD34+ bone marrow cells co-expressing the indicated marker (gated on live singlet CD45+CD34+; positivity defined by fluorescence minus one controls); boxes indicate median and interquartile range: whiskers the 5th–95th percentiles; outliers as open circles; within-patient comparisons used paired tests: donors vs. ALS used unpaired tests

significance [5.3 ± 1.3 (CI 95%: 2.3–8.1) *vs*. 8.2 ± 1.2 (CI 95%: 5.6–10.8), 3.3 ± 1.1 (CI 95%: 0.7–5.8) *vs*. 7.7 ± 3.4 (CI 95%: 0.03–15.4), 3.2 ± 1.2 (CI 95%: 0.5–5.9) *vs*. 2.5 ± 0.6 (CI 95%: 1.2–3.8) at baseline and follow-up, respectively] (Figure 2F–2H). A trend towards a decrease in the level of early B-lymphocyte precursors (CD34+CD10+) in ALS patients was revealed at baseline. At follow-up, there was a tendency to restore the level of CD34+CD10+ with a decrease in the level of later precursors (CD34+CD19+), which is probably associated with the effect of fludarabine on B-lymphocytes [10.9 ± 3.9 (CI 95%: 2.1–19.8) *vs*. 17.2 ± 4.5 (CI 95%: 7.0–27.4) and 13.5 ± 4.1 (CI 95%: 4.2–22.7) *vs*. 12.1 ± 4.3 (CI 95%: 2.5–21.8) at baseline and follow-up, respectively] (Figure 2I and 2J). The mean values of myeloid-leaning markers, particularly CD34+CD117+, CD34+CD90+ CD34+CD133+, CD34+CD33+ and CD34+CD38+ did not undergo significant changes and was defined as [80 ± 3.2 (CI 95%: 72.6–87.1) *vs*. 70.8 ± 8.1 (CI 95%: 52.6–89.1), 28 ± 5.7 (CI 95%: 15.1–41) *vs*. 21.8 ± 3.0 (CI 95%: 15–28.6), 60.2 ± 5.2 (CI 95%: 48.4–72) *vs*. 62.0 ± 4.2 (CI 95%: 52.6–71.5), 47.7 ± 7.7 (CI 95%: 30.2–65.1) *vs*. 53.1 ± 3.4 (CI 95%: 45.5–60.8) and 67.6 ± 6.7 (CI 95%: 52.2–82.6) *vs*. 73.0 ± 5 (CI 95%: 61.6–84.6) at baseline and follow-up, respectively] (Figure 3K–3O).

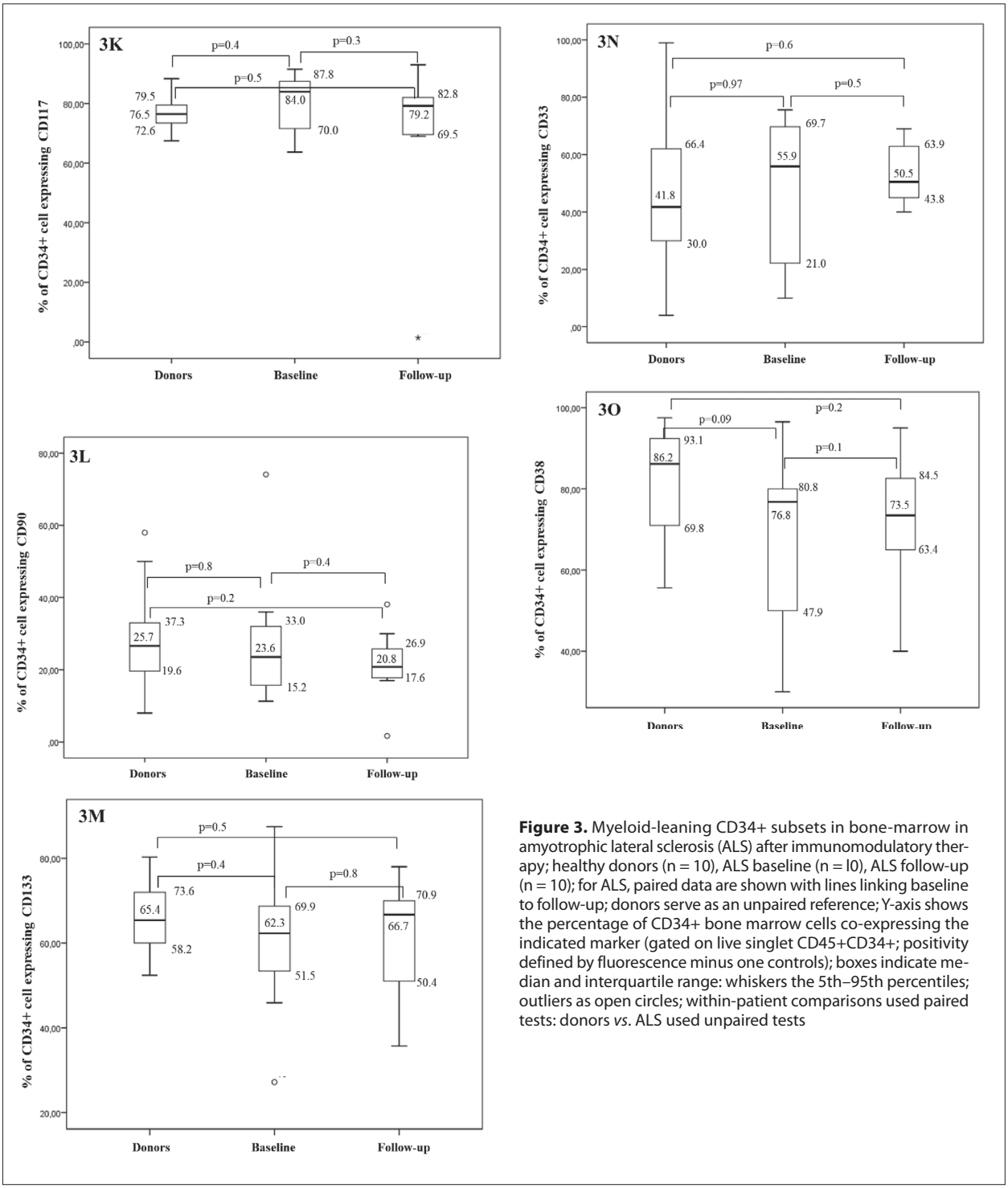


Figure 3. Myeloid-leaning CD34+ subsets in bone-marrow in amyotrophic lateral sclerosis (ALS) after immunomodulatory therapy; healthy donors (n = 10), ALS baseline (n = l0), ALS follow-up (n = 10); for ALS, paired data are shown with lines linking baseline to follow-up; donors serve as an unpaired reference; Y-axis shows the percentage of CD34+ bone marrow cells co-expressing the indicated marker (gated on live singlet CD45+CD34+; positivity defined by fluorescence minus one controls); boxes indicate median and interquartile range: whiskers the 5th–95th percentiles; outliers as open circles; within-patient comparisons used paired tests: donors *vs*. ALS used unpaired tests

DISCUSSION

Uncontrolled or prolonged neuroinflammation is potentially harmful resulting in cellular damage and exacerbates the severity of neurodegenerative diseases such as Parkinson's disease, MS and ALS [12, 13]. This is particularly relevant to neurodegenerative diseases, which are typified by evidence of microglial activation and pro-inflammatory cytokine's oversecreting [7, 14]. Growing evidence suggests that, in addition to microglia, several other subsets of innate immune cells, including macrophages, monocytes, neutrophils, NK cells, and T cells are involved in the pathogenesis of ALS [8, 15]. Evidence has been provided that the overexpression of inflammatory cytokines such as tumor necrosis factor α (TNF-α), IL-1β, IL-2, IL-6, and IL-8 in blood, CSF, and spinal cord tissues are elevated in ALS patients relative to healthy donors [16].

Only single publications have focused on the role of BM as a contributing factor in the spread of neuroinflammation and autoimmune damage [9, 17]. In our study, we found no significant differences in BM CD34+ cell subsets in ALS patients compared to donors. However,

after immunomodulatory therapy there was a significant increase in the CD34+CD13+ and CD34+CD123+ and a decrease in the cells expressing CD34+CD44+ in the patient's BM (Figure 1B–1D).

In BM, the expression of CD123, the alpha chain of IL-3 receptor, is limited to a sub-population of normal stem cells and few lymphoid progenitors. CD123-expressing progenitor cells have increased resistance to apoptosis and high proliferative activity [18]. In humans, IL-3 promotes the activation of plasmacytoid dendritic cells, which are involved in the maintenance of T-cell tolerance [19]. IL-3 suppresses Th17 differentiation and enhances differentiation towards Th2 lymphocytes. In addition, IL-3 promotes the differentiation of naive CD4+CD45RA+ T cells into CD3+CD4+CD25+CD127- regulatory T cells and promote the migration of regulatory T cells by changing in kinase phosphorylation and actin cytoskeleton structure [20]. In Alzheimer's disease (AD), IL3Rα expression in postmortem brain samples is elevated in frontal cortex tissues and correlates with both disease duration and β-amyloid (Aβ) levels. Exposure to IL-3 mediated by CD123+ induces transcriptional, morphological, and functional reprogramming of microglia, endowing them with an acute immune response program, increased motility and the ability to cluster and clear Aβ and tau aggregates [21]. On the other hand, in MS, CD123 is expressed mainly by microglia and recruited myeloid cells in the spinal cord, leading to infiltration by immune cells, increasing the severity of MS [22]. Thus, this discrepancy between AD and MS indicates a dual role of IL-3 in CNS inflammation, when the same mechanism (reprogramming of IL-3R+ myeloid cells) leads to two different consequences (favorable in AD and detrimental in MS).

The transmembrane aminopeptidase CD13 is highly expressed in myeloid lineage cells, hematopoietic progenitors, and stem cells. The CD34+CD13+ subset is found in donor BM and cord blood, reflecting an early stage of human myeloid cell differentiation. The CD13 expression on CD34+ cells precede CD33 expression and is associated with early hematopoietic cells, in the absence of lineage-associated markers [23]. In our observations, BM cells showed no increase in expression of CD117, CD90 and CD133 as well as CD33 and CD38 (Figure 3H–3O).

The adhesion molecule CD44 and its major ligand, hyaluronic acid, play an important role in the migration of normal CD34+ cells [23]. Interestingly that G-CSF-mediated mobilization of stem cells into the peripheral bloodstream results in increased expression of matrix metalloproteinases to which CD13 belongs. As a result, cleavage is activated and CD44 level on BM cells decreases [24, 25]. Our study also demonstrated a negative correlation between the levels of CD34+CD13+ and CD34+CD44+, which may indicate the mobilization of progenitor cells with a certain immunophenotype (probably not expressing CD13) into blood. The detected challenges in the BM may indicate that transfused transdifferentiated *ex vivo* CD34+ HSCs managed to startle out of "steady state" and to start the processes of linear differentiation. We believe that the use of fludarabine and G-CSF were not able to alter BM adhesion molecules (e.g., CD44) due to the fact that the follow-up was carried out for at least five months, which significantly exceeds the period of action of the above-mentioned drugs.

Little is known about the link between CHIP and the risk of neurodegenerative diseases. A recent study found an increased risk of neurodegenerative diseases in patients with *DNMT3A*-mutant CHIP*, ASXL1*-mutant CHIP*, or SRSF2*-mutant CHIP [26]. However, this association does not appear to be unidirectional. Bouzid et al. [27] revealed the causal role for CHIP in reducing AD dementia risk.

Detection of somatic mutations was not the main aim of our study. However, CHIP was detected in three out of 10 ALS patients. After mild immunomodulatory therapy and reinfusion of transdifferentiated autologous CD34+ cells, the mutated clone in the BM fell below detection at the applied coverage. This finding requires a larger patient cohort, a longer follow-up period and the use of NGS technology with high coverage and error correction.

We hypothesized that *ex vivo* manipulated quiescent HSCs may result in restoring a loss of clonal diversity in the entire blood system. It is possible that CHIP was not eliminated, but a reduction in VAF of somatic mutant clones below their detection level at × 100 coverage was achieved. Nevertheless, our data give new look at the problem of controlling unwanted cell clones in BM and peripheral blood to reduce chronic systemic inflammation. Nevertheless, our data provide new insights into the challenge of targeting unwanted cell clones in BM and peripheral blood to reduce chronic systemic inflammation. We have initiated a clinical protocol in ALS patients involving *ex vivo* reinfusion of manipulated autologous CD34+ cells after repeated courses of fludarabine and tocilizumab. The new targeted gene sequencing panel with a higher coverage (× 2000) will provide more information on CHIP dynamics under treatment.

Our hypothesis-generating study has limitations. First, this was a preliminary study investigating the relationship between the CD34+ subsets, CHIP, and the clinical course of ALS; therefore, a power calculation for the sample size was not performed. Consequently, the statistical power may have been insufficient to detect the influence of these factors on therapy outcomes and laboratory findings. Second, the small sample size did not allow us to exclude the random nature of the data obtained in the follow-up period. Third, immune cell subsets were not assessed simultaneously in peripheral blood and, for obvious reasons, in brain and spinal cord tissue.

CONCLUSION

Our study is the first attempt to characterize the subsets of BM HSCs in ALS patients and to reveal changes in their follow-up patterns under the immunomodulatory therapy. Our results have clinical significance, although they are limited and preliminary. First, they demonstrate that BM is one of the organs responding to immune-mediated neuroinflammation. Second, the issue of whether the abnormal

immune response leading to neurodegeneration can be restarted and corrected is raised. In addition, preliminary results indicate a possible link between CHIP and ALS and point the way to eliminating aberrant clones.

Author contributions: Kovalenko NI, Bryukhovetsky AS collected, analyzed and interpreted the clinical data; Shatalov PA contributed to sequencing data collection and carried out the mutation analysis; Grivtsova LY contributed to immunophenotyping data collection, carried out the CD34+ subset analysis, and edited the manuscript; Dolgopolov IS, Rykov MYu. performed the data analyses, wrote the manuscript, supervised and revised the manuscript for intellectual content.

Conflict of Interest: None declared.

REFERENCES

1. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med. 2001;344(22):1688–700. [DOI: 10.1056/NEJM200105313442207] [PMID: 11386269]
2. Gurfinkel Y, Polain N, Sonar K, Nice P, Mancera RL, Rea SL. Functional and structural consequences of TBK1 missense variants in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Neurobiol Dis. 2022;174:105859. [DOI: 10.1016/j.nbd.2022.105859] [PMID: 36113750]
3. Vecellio M, Wu H, Lu Q, Selmi C. The multifaceted functional role of DNA methylation in immune-mediated rheumatic diseases. Clin Rheumatol. 2021;40(2):459–76. [DOI: 10.1007/s10067-020-05255-5] [PMID: 32613397]
4. Evans MA, Walsh K. Clonal hematopoiesis, somatic mosaicism, and age-associated disease. Physiol Rev. 2023;103(1):649–716. [DOI: 10.1152/physrev.00004.2022] [PMID: 36049115]
5. Loh PR, Genovese G, Handsaker RE, Finucane HK, Reshef YA, Palamara PF, et al. Insights into clonal haematopoiesis from 8,342 mosaic chromosomal alterations. Nature. 2018;559(7714):350–5. [DOI: 10.1038/s41586-018-0321-x] [PMID: 29995854]
6. Martin LJ, Adams DA, Niedzwiecki MV, Wong M. Aberrant DNA and RNA Methylation Occur in Spinal Cord and Skeletal Muscle of Human SOD1 Mouse Models of ALS and in Human ALS: Targeting DNA Methylation Is Therapeutic. Cells. 2022;11(21):3448. [DOI: 10.3390/cells11213448] [PMID: 36359844]
7. Yun J, Youn YC, Kim HR. Association Between Clonal Hematopoiesis of Indeterminate Potential and Brain β-Amyloid Deposition in Korean Patients With Cognitive Impairment. Ann Lab Med. 2024;44(6):576–80. [DOI: 10.3343/alm.2024.0086] [PMID: 38802262]
8. Yazdani S, Seitz C, Cui C, Lovik A, Pan L, Piehl F, et al. T cell responses at diagnosis of amyotrophic lateral sclerosis predict disease progression. Nat Commun. 2022;13(1):6733. [DOI: 10.1038/s41467-022-34526-9] [PMID: 36347843]
9. Shi K, Li H, Chang T, He W, Kong Y, Qi C, et al. Bone marrow hematopoiesis drives multiple sclerosis progression. Cell. 2022;185(13):2234-2247.e17. [DOI: 10.1016/j.cell.2022.05.020] [PMID: 35709748]
10. Bryukhovetskiy AS, Grivtsova LY, Sharma HS. Ultra early molecular biologic diagnosis of malignant and neurodegenerative diseases by the immunospecific profiles of the protein's markers of the surface of the mobilized autologous hematopoietic stem cells. Prog Brain Res. 2021;266:75–95. [DOI: 10.1016/bs.pbr.2021.06.002] [PMID: 34689866]
11. Proskurina AS, Gvozdeva TS, Alyamkina EA, Dolgova EV, Orishchenko KE, Nikolin VP, et al. Results of multicenter double-blind placebo-controlled phase II clinical trial of Panagen preparation to evaluate its leukostimulatory activity and formation of the adaptive immune response in patients with stage II-IV breast cancer. BMC Cancer. 2015;15:122. [DOI: 10.1186/s12885-015-1142-z] Erratum in: BMC Cancer. 2016;16:117. [DOI: 10.1186/s12885-016-2163-y] Uchakin, Peter N [added]. [PMID: 25886605]
12. Stacchiotti C, Mazzella di Regnella S, Cinotti M, Spalloni A, Volpe E. Neuroinflammation and Amyotrophic Lateral Sclerosis: Recent Advances in Anti-Inflammatory Cytokines as Therapeutic Strategies. Int J Mol Sci. 2025;26(8):3854. [DOI: 10.3390/ijms26083854] [PMID: 40332510]
13. Zaccai S, Nemirovsky A, Lerner L, Alfahel L, Eremenko E, Israelson A, et al. CD4 T-cell aging exacerbates neuroinflammation in a late-onset mouse model of amyotrophic lateral sclerosis. J Neuroinflammation. 2024;21(1):17. [DOI: 10.1186/s12974-023-03007-1] [PMID: 38212835]
14. Al-Khayri JM, Ravindran M, Banadka A, Vandana CD, Priya K, Nagella P, et al. Amyotrophic Lateral Sclerosis: Insights and New Prospects in Disease Pathophysiology, Biomarkers and Therapies. Pharmaceuticals (Basel). 2024;17(10):1391. [DOI: 10.3390/ph17101391] [PMID: 39459030]
15. McCauley ME, Baloh RH. Inflammation in ALS/FTD pathogenesis. Acta Neuropathol. 2019;137(5):715–30. [DOI: 10.1007/s00401-018-1933-9] [PMID: 30465257]
16. Jiang Z, Wang Z, Wei X, Yu XF. Inflammatory checkpoints in amyotrophic lateral sclerosis: From biomarkers to therapeutic targets. Front Immunol. 2022;13:1059994. [DOI: 10.3389/fimmu.2022.1059994] [PMID: 36618399]
17. Ren H, Liu Q. Skull and vertebral bone marrow in central nervous system inflammation. Fundam Res. 2023;4(2):246–50. [DOI: 10.1016/j.fmre.2023.01.012] [PMID: 38933518]
18. Mani R, Goswami S, Gopalakrishnan B, Ramaswamy R, Wasmuth R, Tran M, et al. The interleukin-3 receptor CD123 targeted SL-401 mediates potent cytotoxic activity against CD34+CD123+ cells from acute myeloid leukemia/myelodysplastic syndrome patients and healthy donors. Haematologica. 2018;103(8):1288–97. [DOI: 10.3324/haematol.2018.188193] [PMID: 29773600]
19. Villadangos JA, Young L. Antigen-presentation properties of plasmacytoid dendritic cells. Immunity. 2008;29(3):352–61. [DOI: 10.1016/j.immuni.2008.09.002] [PMID: 18799143]
20. Ullrich KA, Derdau J, Baltes C, Battistella A, Rosso G, Uderhardt S, et al. IL-3 receptor signalling suppresses chronic intestinal inflammation by controlling mechanobiology and tissue egress of regulatory T cells. Gut. 2023;72(11):2081–94. [DOI: 10.1136/gutjnl-2023-329818] [PMID: 37541770]
21. Chen J, Xu S, Wang L, Liu X, Liu G, Tan Q, et al. Refining the interactions between microglia and astrocytes in Alzheimer's disease pathology. Neuroscience. 2025;573:183–97. [DOI: 10.1016/j.neuroscience.2025.03.033] [PMID: 40120713]
22. Kiss MG, Mindur JE, Yates AG, Lee D, Fullard JF, Anzai A, et al. Interleukin-3 coordinates glial-peripheral immune crosstalk to incite multiple sclerosis. Immunity. 2023;56(7):1502–1514.e8. [DOI: 10.1016/j.immuni.2023.04.013] [PMID: 37160117]
23. Gaipa G, Coustan-Smith E, Todisco E, Maglia O, Biondi A, Campana D. Characterization of CD34+, CD13+, CD33- cells, a rare subset of immature human hematopoietic cells. Haematologica. 2002;87(4):347–56. [PMID: 11940478]
24. Avigdor A, Goichberg P, Shivtiel S, Dar A, Peled A, Samira S, et al. CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34+ stem/progenitor cells to bone marrow. Blood. 2004;103(8):2981–9. [DOI: 10.1182/blood-2003-10-3611] [PMID: 15070674]
25. Hira VVV, Breznik B, Vittori M, Loncq de Jong A, Mlakar J, Oostra RJ, et al. Similarities Between Stem Cell Niches in Glioblastoma and Bone Marrow: Rays of Hope for Novel Treatment Strategies. J Histochem Cytochem. 2020;68(1):33–57. [DOI: 10.1369/0022155419878416] [PMID: 31566074]
26. Liu X, Xue H, Wirdefeldt K, Song H, Smedby K, Fang F, et al. Clonal hematopoiesis of indeterminate potential and risk of neurodegenerative diseases. J Intern Med. 2024;296(4):327–35. [DOI: 10.1111/joim.20001] [PMID: 39073225]
27. Bouzid H, Belk JA, Jan M, Qi Y, Sarnowski C, Wirth S, et al. Clonal hematopoiesis is associated with protection from Alzheimer's disease. Nat Med. 2023;29(7):1662–70. [DOI: 10.1038/s41591-023-02397-2] [PMID: 37160117]

Имунологија *CD*34 субпопулација коштане сржи и клонална хематопоеза неодређеног потенцијала у амиотрофичној латералној склерози

Игор С. Долгополов[1], Људмила Ј. Гривцова[2], Николај И. Коваленко[1], Петар А. Шаталов[3], Андреј С. Брјуховецки[1], Максим Ју. Риков[4,5]

[1]Клиничка болница „Неуровита", Москва, Русија;
[2]Медицински радиолошки научни центар „А. Циб", Калушка област, Русија;
[3]Национални медицински истраживачки радиолошки центар, Одељење молекуларно-генетичке службе, Москва, Русија;
[4]Руски државни социјални универзитет, Москва, Русија;
[5]Централни научноистраживачки институт за организацију и информатизацију здравствене заштите, Москва, Русија

САЖЕТАК

Увод Савремена сазнања о патогенези неуродегенеративних болести указују на то да је инфламација кључни фактор њихове прогресије.

Методе Група са амиотрофичном латералном склерозом обухватила је 10 болесника [просечна старост 55,1 ± 3,1 година (*CI* 95%: 48–62,2)]. Секвенцирање целог егзома и имунофенотипизација *CD34+* субпопулација у коштаној сржи обављени су пре терапије и после шест месеци. Контролну групу је чинило 10 здравих донора, просечне старости 39,9 ± 3,9 година (*CI* 95%: 31,2–48,6).

Резултати Матичне ћелије периферне крви прикупљане су после четвородневне примене фактора стимулације колонија гранулоцита. Просечан број добијених *CD34+* ћелија био је 290,4 ± 53,5 × 10^6 (*CI* 95%: 177,6–403,3). Болесници су примали флударабин у дози од 25 *mg*/m^2 дневно, првог и другог дана. Ради индуковања трансдиференцијације хематопоетских матичних ћелија, матичне ћелије периферне крви су инкубиране *ex vivo* са фрагментима дволанчане ДНК људске плаценте и реинфундоване 48 сати после примене флударабина. Клонална хематопоеза неодређеног потенцијала детектована је у три случаја (30%) пре терапије. Забележено је значајно повећање *CD34+CD13+* и *CD34+CD123+* хематопоетских матичних ћелија у коштаној сржи. Нивои *CD34+CD44+* ћелија значајно су смањени. Нивои *CD34+CD7+*, *CD34+CD2+* и *CD34+CD56+* показали су тренд пораста просечних вредности после третмана. У два случаја, клонална хематопоеза неодређеног потенцијала је нестала, док је у једном случају примећено смањење учесталости варијантног алела. Просечан резултат Ревидиране функционалне скале за амиотрофичну латералну склерозу остао је непромењен [39 ± 0,6 поена (*CI* 95%: 37,6–40,4) наспрам 40,4 ± 0,7 (*CI* 95%: 38,8–42)].

Закључак Наша студија представља први покушај карактеризације субпопулација хематопоетских матичних ћелија коштане сржи код амиотрофичне латералне склерозе. Резултати показују способност коштане сржи да реагује на имунолошки посредовану неуроинфламацију. Прелиминарни резултати указују на могућу везу између клоналне хематопоезе неодређеног потенцијала и амиотрофичне латералне склерозе, отварајући пут ка елиминацији аберантних клонова.

Кључне речи: *CD34* субпопулације; коштана срж; амиотрофична латерална склероза; клонална хематопоеза неодређеног потенцијала; флударабин

DOI: https://doi.org/10.2298/SARH250922084P
UDC: 616.62-006.6

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Correlation of urinary histamine concentration with mast cell density in patients with non-muscle invasive urothelial bladder carcinoma

Mladen Popov[1,2], Tanja Lakić[1,3], Dejan Miljković[1,4], Ivana Isakov[1,5], Ivan Levakov[1,2], Jovo Bogdanović[1,2], Dimitrije Jeremić[1,2], Senjin Đozić[1,2], Stevan Stojanović[1,2], Saša Vojinov[1,2]

[1]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia;
[2]University Clinical Center of Vojvodina, Department of Urology, Novi Sad, Serbia;
[3]University Clinical Center of Vojvodina, Center for Pathology and Histology, Novi Sad, Serbia;
[4]Institute for Pulmonary Diseases of Vojvodina, Center for Pathological-anatomical and Molecular Diagnostics, Sremska Kamenica, Serbia;
[5]University Clinical Center of Vojvodina, Center for Laboratory Diagnostics, Novi Sad, Serbia

SUMMARY

Introduction/Objective There are no reliable tumor markers for non-invasive detection of bladder cancer and monitoring patients after treatment. The role of mast cells in oncogenesis is still unknown.

Methods Our study is an open, longitudinal, prospective follow-up study conducted over six months after surgical treatment of bladder cancer, with preoperative sampling of the first morning urine sample to determine the histamine concentration level. The research included 60 patients of both sexes, aged ≥ 18 years, with the first presentation of a non-muscle invasive bladder cancer. Patients in the study underwent follow-up control urethrocystoscopy postoperatively. The concentration of mast cells in tumor tissue was specified.

Results The study included 35 (58.3%) men and 25 (41.7%) women with an average age of 70.15 ± 9.38 years. The mean urinary histamine levels before surgery in patients with non-muscle-invasive bladder cancer were 11.06 ± 5.79 ng/mL. The mean urinary histamine levels before surgery ($t = 2.46$; $p = 0.02$) and six months after surgery ($t = 2.34$; $p = 0.02$) in patients with T1 stage were statistically significantly higher than the urinary histamine levels in patients with Ta stage of urothelial bladder cancer. Patients with higher histamine concentration in urine before surgery had a higher number of mast cells.

Conclusion The mean urinary histamine value before surgery, three, and six months after surgery is statistically significantly lower than the reference urinary histamine values. The urinary histamine values in patients with T1 stage are higher than in patients with Ta stage. Statistically significant correlation between mast cell concentration and histamine was determined before surgery.

Keywords: bladder cancer; mast cells; histamine; urine

INTRODUCTION

Urothelial bladder cancer is a complex disease characterized by high morbidity and mortality rates despite complex multimodal treatment. The survival of patients with bladder cancer has not significantly increased, and there is still a high rate of tumor recurrence and significant impairment of quality of life [1, 2, 3].

Epidemiologically, bladder cancer is the most common malignant disease of the urinary tract in both sexes [1, 2]. The incidence of bladder cancer is estimated to double by 2040 [1, 2]. In the male population only, bladder cancer is the seventh among the total number of malignant tumors diagnosed worldwide, while in the female population, it ranks 17th globally [2–5]. Among newly detected bladder tumors, 70–75% are non-muscle-invasive, while the remaining 20–25% are muscle-invasive urothelial carcinoma [1, 4]. When considering only the non-muscle-invasive form, about 70% are stage Ta tumors, 20% are stage T1 carcinomas, and 10% are intraepithelial neoplasia or *carcinoma in situ* (CIS) [1, 3, 4].

Although bladder cancer is one of the most common malignant diseases in the human population, there is no reliable tumor marker that would enable early and non-invasive detection and would be applied in monitoring patients after treatment [6, 7].

The results of published research about the role of mast cells in oncogenesis and the biology of malignant tumors are contradictory, especially in urogenital cancers [8–18]. Data indicate that increased mast cell concentrations in tumor tissue are associated with poor prognosis in rectal cancer, melanoma, non-small cell lung cancer, endometrial cancer, multiple myeloma, and Hodgkin lymphoma [6, 7, 9, 10, 15]. At the same time, increased mast cell concentrations in tumors are associated with improved survival in breast cancer, advanced non-small cell lung cancer, and ovarian cancer [8, 9, 15]. In certain malignant tumors, mast cells occur in higher concentrations within the tumor tissue [14], while in other tumors they occur in

Received • Примљено: September 22, 2025
Accepted • Прихваћено: October 25, 2025
Online first: October 30, 2025

Correspondence to:
Mladen POPOV
University Clinical Center of Vojvodina
Department of Urology
Hajduk Veljkova 1
21000 Novi Sad
Serbia
mladen.popov@mf.uns.ac.rs

higher concentrations in the peritumoral tissue [15]. Mast cells are instrumental in immune response and are one of the main sources of histamine [19, 20]. By secreting pro-angiogenic molecules, mast cells also play an important role in angiogenesis [5, 6, 7].

METHODS

This study is an open, longitudinal, prospective follow-up conducted over six months following transurethral resection of the bladder tumor by monopolar resectoscope at the Urology Department of the University Clinical Center of Vojvodina in Novi Sad. The study was conducted from December 1, 2023 to November 1, 2024.

Our research included 60 patients of both sexes, aged ≥ 18 years, with the first presentation of a non-muscle invasive form of urothelial bladder cancer. Preoperative collection of first-morning urine samples was done in order to determine histamine concentration. Patients included in the study were followed up with control urethrocystoscopy three months and six months postoperatively. Before the control urethrocystoscopy, urine samples were collected by spontaneous voiding of the first-morning urine.

The study did not include patients with recurrent bladder tumors, patients with other previously diagnosed malignancy, patients with other concomitant malignancies, as well as patients with allergic or autoimmune diseases and idiopathic mast cell activation syndrome. Individuals receiving antihistamine and/or mast cell stabilizer therapy at the time of enrollment, or within the preceding six months, were excluded from the study.

Patients with pathologically confirmed T2–T4 stage of the disease were excluded from the study, as well those in whom the final pathological examination established the absence of urothelial carcinoma. Patients who did not provide all three planned urine samples were also excluded from the study.

The histological examination of surgically removed tumor tissue included tissue fragments fixed in formalin, molded into paraffin molds and stained with the standard hematoxylin and eosin method. Two additional slides were made, of which, special May-Grünwald Giemsa (MGG) straining was applied to one slide, while immunohistochemical staining (IHC) with the primary monoclonal antibody monocarboxylate transporters (MCT) was performed on the other with the aim of visualizing mast cells in the tumor tissue. Before applying additional stains, a representative paraffin block was selected, and in the case of immunohistochemical analysis, tissue preparation for IHC was performed. After automatic deparaffinization of the bladder tumor sample, the following was done: automated special MGG staining on plate 1 and automated IHC with the monoclonal antibody mast cell tryptase- MCT (monoclonal mouse anti-human clone 10D11, dilution: 1/80, Novocastra Laboratories, Newcastle upon Tyne, United Kingdom) on plate 2. Positive MCT expression represented cytoplasmic staining of tumor cells of any staining intensity. On photographs of 10 fields of view, at 400 × magnification, the surface numerical density of mast cells was analyzed using the Fiji software (ImageJ, LOCI, University of Wisconsin, Madison, WI, USA) program and the Cell Counter function. The mean value was calculated from the number of mast cells per high power field.

To determine urinary histamine concentration, an immunodiagnostic antigens assay (Histamine ELISA, Abcam, Cambridge, United Kingdom) was used. An amount of 10–15 ml of the first-morning urine sample was diluted with reaction buffer in a 1:15 ratio. Then 75 μL of derivatization agent were added to the tube and mixed by repeated inversions. This was followed by a one-hour incubation at room temperature (15–30°C) in a horizontal shaker. After that, 50 μL of histamine antibodies were added and incubated for one hour at room temperature (15–30°C) in a horizontal shaker. The microtiter plate was washed with wash buffer, substrate was added, and incubated for 12–19 minutes at room temperature in the dark. A stop solution was added and the absorbance was immediately determined with an ELISA reader at 450 nm. The obtained histamine concentration values were multiplied by a dilution factor of 15 and expressed in ng/ml. The reference value of histamine in urine is 0.67 ng/ml with a standard derivative of 0.18 ng/ml. The histamine value from the stated unit can be converted to nmol/l using the following formula: histamine (ng/ml) × 8.997 = histamine (nmol/l).

Statistical analysis

Continuous variables were presented as mean ± SD, and differences between groups were analyzed using the student t-test (t) / Mann–Whitney (U). A value of $p < 0.05$ was considered statistically significant. Categorical variables were presented as counts and percentages. Non-parametrically distributed continuous variables were presented using the median, minimum, and maximum values. The relationship between urinary histamine concentration and cancer recurrence was examined using Fisher's exact test. All statistical analyses were performed using SPSS Statistics for Windows, Version 17.0. (SPSS Inc., Chicago, IL, USA).

Ethics: All patients provided written consent prior to their enrollment in the study. The treatment protocol was approved by the Ethics Committee of the University Clinical Centre of Vojvodina (November 16, 2023; No. 600-235). The study was conducted in accordance with the principles of the Declaration of Helsinki of the World Medical Association.

RESULTS

The study included 35 (58.3%) men and 25 (41.7%) women with an average age of 70.15 ± 9.38 years. The average age of the male population was 70.94 years and 69.04 years in female population. An average age of the subjects with Ta stage tumors was 68.87 years, while the average age of the subjects with T1 stage was 72.28 years. Intravesical

Table 1. Demographic and pathohistological characteristics, therapy and recurrence of urothelial bladder carcinoma

Parameters	Frequency	Percentage (%)
Sex		
Male	35	58.3
Female	25	41.7
Type of bladder cancer		
Infiltrative urothelial carcinoma	14	23.3
Papillary urothelial carcinoma	46	76.7
Grade		
High grade	36	60
Low grade	24	40
Stage		
pT1	21	35
pTa	39	65
Macroscopic appearance of the tumor		
Polypoid	23	38.3
Papillary	37	61.7
Tumor base		
Wide	25	41.7
Narrow	35	58.3
Intravesical BCG therapy		
Yes	29	48.3
No	31	51.7
Mitomycin C therapy		
Yes	3	5
No	57	95
Relapse		
Yes	3	5
No	57	95

BCG – Bacillus Calmette–Guérin therapy

Bacillus Calmette–Guérin (BCG) therapy was received by 29 (48.3%) patients, while therapy with Mitomycin C was received by only three (5%) patients. Only three (5%) patients had a recurrence of bladder cancer (Table 1).

The mean urinary histamine levels in patients with Ta and T1 stage are shown in Table 2. The reference urinary histamine levels are 13 ± 4 ng/ml (range 5–21 ng/ml). The mean urinary histamine levels before surgery ($t = -2.59$; $p = 0.01$), three months ($t = -9.26$; $p < 0.001$) and six months ($t = -5.65$; $p < 0.001$) after surgery were statistically significantly lower than the reference values. The mean urinary histamine levels before surgery ($t = 2.46$; $p = 0.02$) and six months after surgery ($t = 2.34$; $p = 0.02$) in patients with T1 stage were statistically significantly higher than the urinary histamine levels in patients with Ta stage of urothelial bladder cancer. The mean age and mast cell count were not statistically significantly different between T1 and Ta stages (Table 2).

A statistically significant weak correlation between mast cell concentration and histamine was found only in the preoperative period ($r = 0.35$; $p = 0.01$). With each increased in histamine concentration (not exceeding the reference values), the concentration of mast cells in bladder

Table 3. Prevalence of pT stage of urothelial bladder carcinoma depending on different variables

Variables		pT Stage		p
		T1	Ta	
Sex	Male	15	20	0.17
		71.4%	51.3%	
	Female	6	19	
		28.6%	48.7%	
Bacillus Calmette–Guérin therapy	Yes	15	14	0.01*
		71.4%	35.9%	
	No	6	25	
		28.6%	64.1%	
		66.7%	28.2%	
Mitomycin C	Yes	1	2	0.99
		4.8%	5.1%	
	No	20	37	
		95.2%	94.9%	
Relapse	Yes	1	2	0.99
		4.8%	5.1%	
	No	20	37	
		95.2%	94.9%	

*$p < 0.05$

Table 4. Incidence of bladder cancer recurrence depending on the urinary histamine concentration

Histamine concentration		Relapse		p
		Yes	No	
Before operation	Lower	3	34	0.28
		100%	59.6%	
	Higher	0	23	
		0%	40.4%	
Three months after operation	Lower	3	52	0.98
		100%	91.2%	
	Higher	0	5	
		0%	8.8%	
Six months after operation	Lower	3	46	0.98
		100%	80.7%	
	Higher	0	11	
		0%	19.3%	

Table 2. Age, urinary histamine concentration, and intratumoral mast cell density in patients with different stages of urothelial bladder cancer

Variables	pT stage				Student t-test (t) / Mann–Whitney test (U)	p
	T1		Ta			
	$\bar{x}$	SD	$\bar{x}$	SD		
Age (years)	72.29	7.29	69.00	10.23	1.30	0.19
Histamine before surgery (ng/ml)	13.47	4.63	9.77	5.99	2.46	0.02*
Histamine – three months after (ng/ml)	7.79	4.66	7.44	4.54	0.29	0.77
Histamine – six months after (ng/ml)	11.38	5.85	8.41	3.95	2.34	0.02*
Mast cell density (number/μm^2)	0.07255	0.02344	0.06256	0.02586	359.50	0.29

*$p < 0.05$

Table 5. Urinary histamine concentration and intratumoral mast cell density depending on implemented intravesical Bacillus Calmette–Guérin (BCR) therapy

Variables	BCG therapy				Student t-test (t) / Mann–Whitney test (U)	p
	Yes		No			
	$\bar{x}$	SD	$\bar{x}$	SD		
Histamine before operation (ng/ml)	12.07	5.27	10.11	6.18	1.32	0.19
Histamine – three months (ng/ml)	6.89	4.29	8.19	4.75	-1.12	0.27
Histamine – six months (ng/ml)	10.15	5.09	8.79	4.64	-1.07	0.28
Mast cell density (number/μm^2)	0.06537	0.02445	0.06669	0.02645	260.50	0.58

cancer tissue also increases. There was no statistically significant correlation between mast cell concentration and histamine concentration three and six months after surgery (Table 4).

The use of BSG therapy was statistically significantly associated with the stage of the disease (Fisher's exact test = 6.90; p = 0.01) with a weak correlation coefficient (φ = 0.34; p = 0.01). Patients who received BCG therapy were more often in the pT1 stage. No statistically significant association was found between sex, use of Mitomycin C, and the recurrence with the pT stage of bladder cancer (Table 3).

Histamine concentrations in urine during the examination were divided according to the mean values: lower histamine values (< 13 ± 4 ng/ml) and higher histamine values (≥ 13 ± 4 ng/ml). Histamine concentrations before surgery (Fisher's exact test = 1.96; p = 0.28), three months after surgery (Fisher's exact test = 0.28; p = 0.98) and six months after surgery (Fisher's exact test = 0.71 p = 0.98) were not statistically associated with bladder cancer recurrence (Table 4).

Comparing the concentration of histamine in urine before surgery, three and six months after surgery, as well as the number of mast cells in patients with and without BCG therapy, no statistically significant differences were found (Table 5).

DISCUSSION

Considering the prevalence and incidence of urothelial bladder cancer worldwide [1, 2], our research is dedicated to improving the existing knowledge about non-muscle invasive bladder cancer.

Previous studies have shown a higher incidence of bladder cancer in males than in females [1, 2]. The sex and the age distribution of the subjects in our study is consistent with previous studies [1, 2, 21, 22]. No sex differences were found in overall survival after treatment of non-muscle invasive bladder cancer [21, 22]. According to current data, the average age of patients with non-muscle invasive bladder cancer is 73 years [1, 2, 21, 22]. The average age of the subjects in our study was 70.15 years. The average age of the male population of subjects is 70.94 years, and the average age of female subjects was 69.04 years. The results of our study are in agreement with the results published by other researchers [1, 2, 21, 22]. Our study found no statistically significant difference in the average age of Ta and T1 stage groups. Literature data indicate that older patients are more likely to develop high-grade tumors and multifocal disease with a higher rate of recurrence and disease progression compared to those younger than 65 years of age [22, 23, 24].

The available results on the role of mast cells in carcinogenesis are contradictory, especially in the case of bladder tumors [12, 15–19]. Authors have found a positive correlation between the concentration of mast cells in the *lamina propria* of the mucosa, the degree of tumor differentiation, and the pathological grade in bladder cancer [13, 25]. A review of the existing literature found a small number of published scientific research results that examined the role of mast cells in urothelial bladder cancer [13, 16, 17, 18].

Dowell et al. [25] found that the most abundant Interleukin-17-positive cells in bladder tumors are mast cells. In patients with primary and concomitant CIS who received intravesical BCG immunotherapy, higher Interleukin-17+ cell counts were associated with improved event-free survival [25].

The results of the study by Popov et al. [26] indicated a positive relationship between mast cell concentration in tumor tissue and the cancer recurrence, but did not detect a relationship between mast cell concentration and the time of recurrence, or a relationship with tumor stage and grade, sex, and age of patients. Our results are consistent with the data from the study of Popov et al. [26].

Simsekoglu et al. [27] were the first who examined the predictive value of mast cells in patients who underwent surgery for non-muscle invasive bladder cancer and then received intravesical immunotherapy with BCG. This study found that histamine concentration significantly increased after the start of intravesical BCG instillation, but no correlation was found between tumor stage, urinary histamine concentration, and the presence of a local response to BCG instillation [27]. In our research the concentration of histamine before surgery and during follow-up was not statistically associated with bladder cancer recurrence nor was it related to BCG therapy. The obtained results are in agreement with the results of Simsekoglu et al. [27].

Our results show that the mean urinary histamine levels before surgery and six months after surgery in patients with T1 stage were statistically significantly higher than the urinary histamine levels in patients with Ta stage of urothelial bladder cancer. The results indicate that a correlation can still be established between the stage of the bladder tumor and urinary histamine levels, although histamine levels in patients with bladder cancer are still lower than the reference values. Researchers have found that the T1 tumor population is very heterogeneous [28, 29]. It is assumed that the same density of tumor infiltration by mast cells, depending on the number and type of existing genetic mutations of urothelial cells, causes different degrees of malignant transformation in different individuals [28,

29]. In our case mast cell density did not differ statistically significantly between T1 and Ta stages.

A study conducted by Sari et al. [28], with 78 subjects, found a statistically significant correlation between the concentration of mast cells in tumor tissue, tumor grade, and stage of the disease ($p < 0.05$; $r = 0.69$ and 0.63). It was found that a higher concentration of mast cells in bladder cancer tissue was statistically significantly associated with a higher density of capillary blood vessels per unit volume of malignant tumor ($p < .05$, $r = 0.56$). Interestingly, the tumor concentration of mast cells in the bladder mucosa in the immediate vicinity of the tumor was higher compared to the concentration of mast cells in the tumor tissue itself ($p < 0.001$) [28]. There are assumptions that the surrounding tissue, which is not microscopically malignantly transformed, plays a key role in the survival of neoplastic tissue [13–17, 28, 29].

While the predictive role of the initial concentration of tumor mast cell infiltration and patient survival after radical cystectomy has been established in muscle-invasive urothelial bladder carcinoma [29], this correlation has not been clarified in the non-muscle-invasive form. Patients with a higher concentration of mast cells in the tumor stroma before radical cystectomy have been found to have a statistically significantly worse overall survival and recurrence-free survival compared to patients with a lower concentration [29].

Findings reported by Sari et al. [28] were in agreement with our results, as no statistically significant correlation between the concentration of mast cells in the bladder tumor tissue, tumor stage and grade was established, observing and comparing the concentration of mast cells in Ta, T1, and T2 stage tumors, although T2 tumors showed the highest concentration of mast cells in tumor tissue [28]. In our study, observing the mean value of mast cell density, it was found that the mast cells density in pTa tumor tissue was lower compared to the number of mast cells in tumor tissue at the T1 stage of the disease (0.06 *vs.* 0.07).

Histamine concentration in blood and urine can be affected by numerous factors [30]. Researchers have found that the bladder mucosa of patients with interstitial cystitis contains an increased concentration of mast cells, while the urine of these patients contains an increased concentration of methylhistamine, which is the main metabolite of histamine [30].

The primary limitation of our study was the relatively short duration of patient follow-up. An additional limitation was the number of subjects in the study. The sample of subjects was relatively small, but it was in correlation or even larger than the number of subjects in previously conducted studies. In most studies, subjects with muscle-invasive cancer were also included.

CONCLUSIONS

The mean urinary histamine value before surgery, three and six months after surgery is statistically significantly lower than the reference urinary histamine values. The mean urinary histamine values before surgery and six months after surgery in patients with T1 stage are statistically significantly higher than the urinary histamine concentration in patients with Ta stage. Histamine concentration before surgery and during follow-up was not statistically associated with bladder cancer recurrence.

There is no statistically significant difference in terms of the number of mast cells in the tumor tissue between Ta and T1 stages. A statistically significant correlation between mast cell concentration and histamine was determined before surgery but not in follow-up. With each increase in histamine concentration, the concentration of mast cells in bladder cancer tissue also increased.

Comparing the concentration of histamine in urine before surgery and during follow-up, as well as the number of mast cells in patients with and without BCG therapy, no statistically significant differences were found.

ACKNOWLEDGMENT

Results of this paper are part of the doctoral dissertation of the first author of the paper.

Conflict of interest: None declared.

REFERENCES

1. Jubber I, Ong S, Bukavina L, Black PC, Compérat E, Kamat AM, et al. Epidemiology of Bladder Cancer in 2023: A Systematic Review of Risk Factors. Eur Urol. 2023;84(2):176–90. [DOI: 10.1016/j.eururo.2023.03.029] [PMID: 37198015]
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. [DOI: 10.3322/caac.21660] [PMID: 33538338]
3. Radovanović M, Petrović M, Šantrić V, Zubelić A. Predictors of response to BCG therapy in non-muscle invasive bladder cancer. Srp Arh Celok Lek. 2022:150(1–2):113–6. [DOI: 10.2298/SARH210928009R]
4. Maas M, Todenhöfer T, Black PC. Urine biomarkers in bladder cancer – current status and future perspectives. Nat Rev Urol. 2023;20(10):597–614. [DOI: 10.1038/s41585-023-00773-8] [PMID: 37225864]
5. Noto CN, Hoft SG, DiPaolo RJ. Mast Cells as Important Regulators in Autoimmunity and Cancer Development. Front Cell Dev Biol. 2021;9:752350. [DOI: 10.3389/fcell.2021.752350] [PMID: 34712668]
6. Sakita JY, Elias Oliveira J, Carlos D, de Souza Santos E, Almeida LY, Malta TM, et al. Mast cell-T cell axis alters development of colitis-dependent and colitis-independent colorectal tumours: potential for therapeutically targeting via mast cell inhibition. J Immunother Cancer. 2022;10(10):e004653. [DOI: 10.1136/jitc-2022-004653] [PMID: 36220303]
7. Fan F, Gao J, Zhao Y, Wang J, Meng L, Ma J, et al. Elevated Mast Cell Abundance Is Associated with Enrichment of CCR2+ Cytotoxic T Cells and Favorable Prognosis in Lung Adenocarcinoma. Cancer Res. 2023;83(16):2690–703. [DOI: 10.1158/0008-5472.CAN-22-3140] [PMID: 37249584]
8. Leveque E, Rouch A, Syrykh C, Mazières J, Brouchet L, Valitutti S, et al. Phenotypic and Histological Distribution Analysis Identify Mast Cell Heterogeneity in Non-Small Cell Lung Cancer. Cancers (Basel). 2022;14(6):1394. [DOI: 10.3390/cancers14061394] [PMID: 35326546]

9. Benito Martin A, Nogués L, Hergueta Redondo M, Castellano Sanz E, Garvin E, Cioffi M, et al. Mast cells impair melanoma cell homing and metastasis by inhibiting HMGA1 secretion. Immunology. 2023;168(2):362–73. [DOI: 10.1111/imm.13604] [PMID: 36352838]
10. Han S, Jiang D, Zhang F, Li K, Jiao K, Hu J, et al. A new immune signature for survival prediction and immune checkpoint molecules in non-small cell lung cancer. Front Oncol. 2023;13:1095313. [DOI: 10.3389/fonc.2023.1095313] [PMID: 36793597]
11. Song X, Jiao J, Qin J, Zhang W, Qin W, Ma S. Single-cell transcriptomics reveals the heterogeneity and function of mast cells in human ccRCC. Front Immunol. 2025;15:1494025. [DOI: 10.3389/fimmu.2024.1494025] [PMID: 39840068]
12. Hempel Sullivan H, Heaphy CM, Kulac I, Cuka N, Lu J, Barber JR, et al. High Extratumoral Mast Cell Counts Are Associated with a Higher Risk of Adverse Prostate Cancer Outcomes. Cancer Epidemiol Biomarkers Prev. 2020;29(3):668–75. [DOI: 10.1158/1055-9965.EPI-19-0962] [PMID: 31932412]
13. Liu Z, Huang C, Mao X, Mi J, Zhang Q, Xie Y, et al. Single cell RNA-Sequencing Reveals Mast Cells Enhance Mononuclear Phagocytes Infiltration in Bladder Cancer Microenvironment. J Cancer. 2024;15(17):5672–90. [DOI: 10.7150/jca.99554] [PMID: 39308672]
14. Song X, Jiao J, Qin J, Zhang W, Qin W, Ma S. Single-cell transcriptomics reveals the heterogeneity and function of mast cells in human ccRCC. Front Immunol. 2025;15:1494025. [DOI: 10.3389/fimmu.2024.1494025] [PMID: 39840068]
15. Pan Q, Wang L, Chai S, Zhang H, Li B. The immune infiltration in clear cell Renal Cell Carcinoma and their clinical implications: A study based on TCGA and GEO databases. J Cancer. 2020;11(11):3207–15. [DOI: 10.7150/jca.37285] [PMID: 32231726]
16. Fu Y, Sun S, Bi J, Kong C, Yin L. Construction and analysis of a ceRNA network and patterns of immune infiltration in bladder cancer. Transl Androl Urol. 2021;10(5):1939–55. [DOI: 10.21037/tau-20-1250] [PMID: 34159075]
17. Li Y, Liu Y, Kang Z, Guo J, Liu N. Tumor microenvironment heterogeneity in bladder cancer identifies biologically distinct subtypes predicting prognosis and anti-PD-L1 responses. Sci Rep. 2023;13(1):19563. [DOI: 10.1038/s41598-023-44028-3] [PMID: 37949863]
18. Choi HW, Naskar M, Seo HK, Lee HW. Tumor-Associated Mast Cells in Urothelial Bladder Cancer: Optimizing Immuno-Oncology. Biomedicines. 2021;9(11):1500. [DOI: 10.3390/biomedicines9111500] [PMID: 34829729]
19. Aponte Lopez A, Munoz Cruz S. Mast Cells in the Tumor Microenvironment. Adv Exp Med Biol. 2020;1273:159–73. [DOI: 10.1007/978-3-030-49270-0_9] [PMID: 33119881]
20. Theoharides TC. The Role of Mast Cells and Their Inflammatory Mediators in Immunity. Int J Mol Sci. 2023;24(15):12130. [DOI: 10.3390/ijms241512130] [PMID: 37569506]
21. Theodorescu D, Li Z, Li X. Sex differences in bladder cancer: Emerging data and call to action. Nat Rev Urol. 2022;19(18):447–79. [DOI: 10.1038/s41585-022-00591-4] [PMID: 35379942]
22. Lin W, Pan X, Zhang C, Ye B, Song J. Impact of Age at Diagnosis of Bladder Cancer on Survival: A Surveillance, Epidemiology, and End Results-Based Study 2004–2015. Cancer Control. 2023;30:10732748231152322. [DOI: 10.1177/10732748231152322] [PMID: 36662642]
23. Ribatti D, Tamma R, Vacca A. Mast Cells and Angiogenesis in Human Plasma Cell Malignancies. Int J Mol Sci. 2019;20(3):481. [DOI: 10.3390/ijms20030481] [PMID: 30678047]
24. Witjes JA, Bruins HM, Cathomas R, Comperat EM, Cowan NC, Gakis G, et al. European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. Eur Urol. 2021;79(1):82–104. [DOI: 10.1016/j.eururo.2020.03.055] [PMID: 32360052]
25. Dowell AC, Cobby E, Wen K, Devall AJ, During V, Anderson J, et al. Interleukin-17-positive mast cells influence outcomes from BCG for patients with CIS: Data from a comprehensive characterisation of the immune microenvironment of urothelial bladder cancer. PLoS One. 2017;12(9):e0184841. [DOI: 10.1371/journal.pone.0184841] [PMID: 28931051]
26. Popov H, Kobakova I, Stoyanov GS, Softova E, Ghenev P. Quantitative Analysis of Tumor-Associated Mast Cells in Recurrent and Non-recurrent Urothelial Bladder Cancer in Stage pTa and pT1. Cureus. 2021;13(4):e14311. [DOI: 10.7759/cureus.14311] [PMID: 33968521]
27. Simsekoglu MF, Kaleler I, Onal B, Demirdag C, Citgez S, Uslu E, et al. Do urinary mast cell mediators predict immune response to BCG in patients with primary high-grade non-muscle invasive bladder cancer? Int J Clin Pract. 2021;75(5):e13959. [DOI: 10.1111/ijcp.13959] [PMID: 33369059]
28. Sari A, Calli A, Cakalagaoglu F, Altınboga AA, Bal K. Association of mast cells with microvessel density in urothelial carcinomas of the urinary bladder. Ann Diagn Pathol. 2012;16(1):1–6. [DOI: 10.1016/j.anndiagpath.2011.07.001] [PMID: 22018898]
29. Liu Z, Zhu Y, Xu L, Zhang J, Xie H, Fu H, et al. Tumor stroma-infiltrating mast cells predict prognosis and adjuvant chemotherapeutic benefits in patients with muscle invasive bladder cancer. Oncoimmunology. 2018;7(9):e1474317. [DOI: 10.1080/2162402X.2018.1474317] [PMID: 30393586]
30. El Mansoury M, Boucher W, Sant GR, Theoharides TC. Increased urine histamine and methylhistamine in interstitial cystitis. J Urol. 1994;152(2 Pt 1):350–3. [DOI: 10.1016/s0022-5347(17)32737-4] [PMID: 8015069]

Повезаност концентрације хистамина у урину са густином мастоцита код болесника са немишићно-инвазивним уротелним карциномом мокраћне бешике

Младен Попов[1,2], Тања Лакић[1,3], Дејан Миљковић[1,4], Ивана Исаков[1,5], Иван Леваков[1,2], Јово Богдановић[1,2], Димитрије Јеремић[1,2], Сењин Ђозић[1,2], Стеван Стојановић[1,2], Саша Војинов[1,2]

[1]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[2]Универзитетски клинички центар Војводине, Клиника за урологију, Нови Сад, Србија;
[3]Универзитетски клинички центар Војводине, Центар за патологију и хистологију, Нови Сад, Србија;
[4]Институт за плућне болести Војводине, Служба за патолошко-анатомску и молекуларну дијагностику, Сремска Каменица, Србија;
[5]Универзитетски клинички центар Војводине, Центар за лабораторијску дијагностику, Нови Сад, Србија

САЖЕТАК

Увод/Циљ Не постоје поуздани туморски маркери који би омогућили неинвазивно откривање карцинома мокраћне бешике и који би се примењивали у праћењу болесника после лечења. Улога мастоцита у процесу онкогенезе и даље је неразјашњена.

Методе Спроведена је отворена, лонгитудинална, проспективна студија са шестомесечним праћењем болесника после хируршког лечења карцинома мокраћне бешике, уз преоперативно узорковање првог јутарњег урина ради одређивања концентрације хистамина. Истраживање је обухватило 60 болесника оба пола, старости ≥ 18 година, са првом презентацијом немишићно-инвазивног карцинома мокраћне бешике. Болесници у истраживању су праћени контролним уретроцистоскопским прегледима. Утврђена је густина мастоцита у туморском ткиву.

Резултати Студија је обухватила 35 (58,3%) мушкараца и 25 (41,7%) жена са просечном старошћу од 70,15 ± 9,38 година. Просечни нивои хистамина у урину пре операције код болесника са немишићно-инвазивним карциномом мокраћне бешике били су 11,06 ± 5,79 *ng/ml*. Просечни нивои хистамина у урину пре операције ($t = 2,46$; $p = 0,02$) и шест месеци после операције ($t = 2,34$; $p = 0,02$) код болесника са стадијумом *T1* били су статистички значајно виши од нивоа хистамина у урину код болесника са стадијумом *Ta* уротелног карцинома мокраћне бешике. Болесници са вишом концентрацијом хистамина у урину пре операције имали су већи број мастоцита.

Закључак Просечна вредност хистамина у урину пре операције, три и шест месеци после операције статистички је значајно нижа од референтних вредности хистамина у урину. Просечне вредности хистамина у урину код болесника са стадијумом *T1* статистички су значајно веће од концентрације хистамина у урину код болесника са стадијумом *Ta*. Статистички значајна корелација између концентрације мастоцита и хистамина утврђена је пре операције.

Кључне речи: карцином мокраћне бешике; мастоцити; хистамин; урин

DOI: https://doi.org/10.2298/SARH251031090M
UDC: 616.718.4-001.5

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Influence of femoral shaft fracture extension into subtrochanteric or supracondylar region on operation and fluoroscopy time in dynamic extramedullary fixation

Milan M. Mitković[1,2], Saša S. Milenković [1,2], Predrag M. Stojiljković[1,2], Mladen J. Stojanović[1], Nikola J. Kostić[1], Miloš A. Nagorni[1]

[1]University Clinical Center Niš, Academician Prof. Dr. Milorad Mitković Clinic for Orthopedics and Traumatology, Niš, Serbia;
[2]University of Niš, Faculty of Medicine, Niš, Serbia

SUMMARY

Introduction/Objective Although femoral shaft fractures are mostly treated by intramedullary fixation today, certain situations indicate extramedullary fixation for these fractures. The aim of this study was to evaluate the influence of femoral shaft fracture extension into the subtrochanteric or supracondylar region on operation time and intraoperative fluoroscopy time while performing dynamic extramedullary fixation.

Method A total of 90 cases of femoral shaft fractures treated using Selfdynamizable Internal Fixator (SIF) were analyzed. Patients were divided into three groups according to the applied implant type: femoral shaft fractures with proximal extension (SIF-troch), femoral shaft fractures without proximal or distal extension (SIF-shaft), and femoral shaft fractures with distal extension (SIF-cond).

Results The shortest mean operation time was observed in the SIF-shaft group, while the longest was recorded in fractures extending into the supracondylar region (SIF-cond group). The shortest average fluoroscopy time occurred in the SIF-shaft group, while the longest in the group with the fracture extension into the subtrochanteric region (SIF-troch group). Operation time was mainly influenced by the technique of lag screws and locking screws insertion and by the fracture reduction maintenance in the subtrochanteric and supracondylar regions.

Conclusion Extension of femoral shaft fractures into the subtrochanteric or supracondylar region is associated with increased operation and fluoroscopy times. SIF allows for dynamic extramedullary fixation, but also provides a relatively simple and fast performing surgical technique for femoral shaft fractures fixation, especially when the fracture does not extend beyond the shaft area.

Keywords: Selfdynamizable Internal Fixator; shaft; fluoroscopy

INTRODUCTION

Closed femoral shaft fractures are today most often treated by intramedullary fixation [1–5]. However, extramedullary fixation has also its place in the treatment of these fractures [6–10]. Extramedullary fixation would be preferred, or the only feasible option of internal fixation, in the following situations of a femoral shaft fracture: narrow medullary canal, closed or deformed medullary canal (after fracture healing, after intramedullary nail removal, etc.), intramedullary presence of an implant (endoprosthesis, nail, screw, etc.), some comminuted fractures requiring better control of length and rotation, pathologic fractures (reaming and insertion of the nail can lead to comminution of weakened bone; reaming can lead to dissemination of malignant cells), extremely obese patients (reaming and pin insertion can be technically challenging), patients with severe cardiorespiratory diseases (reaming can increase the risk of embolism), etc. [9, 10]. Denisiuk et al. [10] reported that extramedullary fixation is recommended for femoral shaft fractures extending into the proximal or distal femur, where intramedullary fixation may be contraindicated.

Extramedullary fixation of femoral shaft fractures with plates is generally accompanied by higher risk of mechanical complication (implant failure/loosening) [11, 12]. High bending forces acting on a rigid extramedullary implant (such as a plate) may be the main causal factor for these complications and it could be considered as a reason why the fixation of femoral shaft fractures is more often being performed today by an intramedullary nailing. In order to transform implant bending forces as more as possible into translational and compression forces between fracture fragments (compression stimulates the fracture healing), a special type of extramedullary implant – Selfdynamizable Internal Fixator (SIF) was developed. This implant provides an initially rigid fixation, with the feature of subsequent spontaneous transition into a dynamic mode, reducing the implant load, and thereby its risk of bending or breaking [13–16]. Delayed dynamization has been considered a desirable

Received • Примљено: October 31, 2025
Accepted • Прихваћено: November 26, 2025
Online first: November 28, 2025

Correspondence to:
Milan M. MITKOVIĆ
Romanijska 19/52
18000 Niš
Serbia
milanmitkovic@hotmail.com

factor in promoting healing of the shaft fractures, especially in delayed-union [15, 17, 18]. Thus, wider use of a such dynamic implants could increase the prevalence of extramedullary fixation in femoral shaft fractures treatment, and there is the need to examine its clinical aspects, both intraoperatively and postoperatively.

Femoral fracture extending from the shaft region into the adjacent area proximally (subtrochanteric region) or distally (supracondylar region) requires the applied extramedullary fixation implant to be longer [10]. These implants contain screws not only for the shaft but also for the proximal or distal region of the femur.

The aim of this study was to examine the effect of femoral shaft fractures extending into the subtrochanteric or supracondylar region on the operation time and the intraoperative fluoroscopy time, as well as the relationship between these parameters.

METHODS

A total of 90 patients treated surgically for a femoral shaft fracture were analyzed in this study. The fixation was performed in all patients using SIF, a specific type of extramedullary implant for dynamic fixation. There were three groups of patients: SIF-troch group included 22 patients with femoral shaft fracture extending into subtrochanteric region, SIF-shaft included 35 patients with femoral shaft fracture without extending into any adjacent area, and SIF-cond group included 33 patients with femoral shaft fracture extending into supracondylar region (Table 1). In the first group, patients were treated by SIF type containing a "trochanteric unit", through which sliding screws for the femoral neck and head are inserted. Patients from the second group were treated by the type of SIF that did not contain any additional unit. In patients from the third group, a SIF with the "condylar unit" had been used, involving locking screws for the femoral condylar region, being locked in the threads of this unit. The main part of the SIF implant is cylindrical, providing axial sliding and rotational contact with the clamps. Some screws pass through the clamps, and when these screws are fully tightened, the clamps are rigidly fixed to the cylindrical part. At one end of the fixator, there is a dynamic slot through which a dynamic anti-rotation screw is inserted. This assembly allows for initially rigid fixation of fractures in the shaft, subtrochanteric, and supracondylar region. However, it also permits that, if biomechanical forces loosen the contact between the screw and the clamp, spontaneous transformation into dynamic fixation occurs, allowing compression at the fracture site and thereby stimulating the healing process. In addition to dynamic anti-rotation screw, the SIF-shaft (type of SIF without additional "unit") at the other end contains a static anti-rotation screw. The number of clamps may vary depending on the surgeon's assessment (Figure 1) [13, 14].

There are SIF implants of different lengths. For fractures extending proximally or distally from the shaft region, SIF-troch and SIF-cond implants with lengths of 250 mm and 300 mm are used SIF-troch and SIF-cond implants of 150 mm or 200 mm are used only for fractures that do not extend from the subtrochanteric or supracondylar region into the femoral shaft). Therefore, only the patients with these longer implant lengths were analyzed in this study.

Table 1. Distribution of patients and their age

Group	Patients (n)			Age [mean ± SD (min–max)] (years)
	Male	Female	Total	
SIF-troch	9	13	22	69.6 ± 14.5 (39–89)
SIF-shaft	11	24	35	69.9 ± 17 (22–91)
SIF-cond	8	25	33	73.2 ± 11.9 (23–90)
Total	28	62	90	71.0 ± 14.6 (22–91)

SIF-troch – femoral shaft fractures with proximal extension; SIF-shaft – femoral shaft fractures without proximal or distal extension; SIF-cond – femoral shaft fractures with distal extension

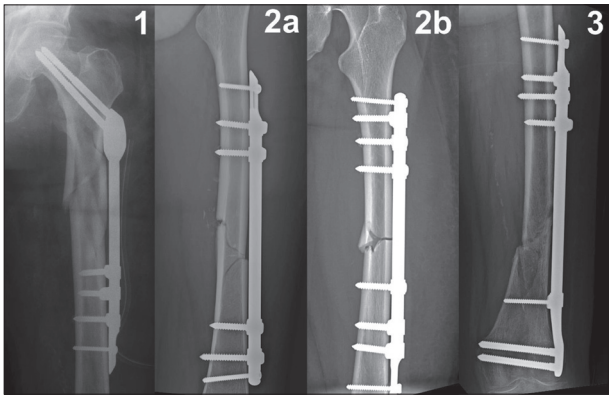


Figure 1. Types of Selfdynamizable Internal Fixator (SIF) used in fixation of femoral shaft fractures; 1 – SIF-troch (containing trochanteric unit in the implant) in the fracture extending into the subtrochanteric region; 2a, 2b – SIF-shaft (without additional unit in the implant) in fractures confined to the shaft region, with different SIF orientations and different numbers of clamps; 3 – SIF-cond (containing condylar unit in the implant) in the fracture extending into the supracondylar region

The values of operation time (minutes) and intraoperative fluoroscopy time (seconds) were analyzed among the groups, as well as the correlation between these parameters, for consecutive patients with available data, treated over a three-year period between 2022 and 2025. Operation time was defined as the time from initial skin incision to the wound suture completion. Statistical analysis was performed by IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA) using t-test and Mann–Whitney U test (to compare values), and Pearson's and Spearman's correlation coefficients (to analyze bivariate correlation). The level of significance set at $p < 0.05$.

Ethics: The study was performed in line with the Declaration of Helsinki and approved by the Ethics Board of the University Clinical Center Niš (Decision No. 29879).

RESULTS

The average operation time was the shortest in SIF-shaft group (where the fracture did not extend beyond the shaft region), while it was the longest in SIF-cond group. The

Table 2. Average values of operation time and fluoroscopy time in the groups (mean ± SD), and parameters of their correlation

Group	Operation time (minutes)	Fluoroscopy time (seconds)	Correlation
SIF-troch	83.4 ± 18.7	44.5 ± 14.2	p = 0.139 r = 0.325
SIF-shaft	76.1 ± 13.9	16.3 ± 6.9	p = 0.012 rs = 0.418
SIF-cond	91.2 ± 21.3	34.2 ± 14	p = 0.104 r = 0.288

SIF-troch – femoral shaft fractures with proximal extension; SIF-shaft – femoral shaft fractures without proximal or distal extension; SIF-cond – femoral shaft fractures with distal extension

Table 3. Comparison of operation time and fluoroscopy time between the groups

Compared groups	Operation time	Fluoroscopy time
SIF-shaft, SIF-troch	p = 0.152*	p < 0.001*
SIF-shaft, SIF-cond	p = 0.001*	p < 0.001*
SIF-troch, SIF-cond	p = 0.001*	p = 0.010**

SIF-troch – femoral shaft fractures with proximal extension; SIF-shaft – femoral shaft fractures without proximal or distal extension; SIF-cond – femoral shaft fractures with distal extension;
*Mann–Whitney U Test;
***t*-test

average fluoroscopy time was also shortest in SIF-shaft group, but longest in SIF-troch group (Table 2). The difference among the groups in terms of both operation time and fluoroscopy time was confirmed in all cases ($p < 0.05$), except for the comparison of operation times between SIF-shaft and SIF-troch groups ($p > 0.05$) (Table 3). A positive correlation between operation time and fluoroscopy time was confirmed in SIF-shaft group ($p < 0.05$), whereas in the other groups the correlation did not reach statistical significance but was close to ($p < 0.2$) (Table 2). The groups did not differ significantly with respect to gender or age distribution ($p > 0.05$).

DISCUSSION

In patients with fractures confined to the femoral shaft (SIF-shaft group), only shaft screws were used. Placement of these screws does not require strict fluoroscopic control during the positioning of each screw. If the second cortex is felt while screwing, just a single fluoroscopy after several screws placement could be sufficient. Furthermore, reduction of the fractures confined to the femoral shaft can be achieved quite easily while applying the SIF-shaft implant. The procedure begins with setting the rotation of the distal fragment through positioning on the traction table. Then two peripheral antirotational screws are placed, followed by control of the fracture angulation in coronal and sagittal planes using bone-holding forceps while the remaining screws (screws for clamps) are inserted [14, 15]. The open technique does not necessarily require a single long incision; it can also be performed through the two smaller incisions, one of which includes both the fracture site and the screws at one end of the implant [19]. This can explain why, in fractures confined to the femoral shaft, both the operative time and fluoroscopy time were the shortest. Since this approach provides good visual control of the fracture alignment, it also explains why the variability of the operative time was minimal and correlated to the fluoroscopy time.

Fixation of the femoral shaft fractures extending into the subtrochanteric region was performed in this study by the SIF type containing sliding screws for the proximal femur (SIF-troch). Sliding screws placement requires more fluoroscopic controls to prevent protrusion of the sliding screw outside the femoral neck and head [13, 15]. Since the proximal parts of the sliding screws are not directly visible intraoperatively, fluoroscopic verification is often repeated, resulting in the longest fluoroscopy time among the patient groups. Longer fluoroscopy time in this group is also influenced by the need for careful and occasionally challenging control of fracture angulation in proximal part while performing extramedullary fixation. The unconfirmed correlation between operative time and fluoroscopy time in this patient group could be explained by shorter duration of surgical steps that require frequent fluoroscopic verification (sliding screws setting) compared to the other steps of the surgery that do not involve frequent imaging (e.g., placement of the shaft screws and wound closure). For this reason, it could be considered that there was no significant difference in average operative times between the SIF-shaft and SIF-troch groups.

Operative time was longest in femoral shaft fractures extending into the supracondylar region, likely due to the difficulty of reduction, as the hamstrings tend to pull the fracture into recurvatum [11, 20]. Consequently, a traction table was not used in most patients in this study, further complicating the reduction and prolonging the surgery. Placement of distal locking screws in the condylar region requires fluoroscopic verification, sometimes repeated, explaining the longer fluoroscopy time compared to the SIF-shaft group. Nevertheless, inserting these screws usually requires fewer repeated fluoroscopic checks than inserting the sliding screws for the proximal femur, which may explain the shorter fluoroscopy time in the SIF-cond group compared to the SIF-troch group.

Concerning extramedullary fixation of femoral shaft fractures, few data are available in the literature regarding operation time and fluoroscopy time, and these mostly refer to the plate fixation. Park et al. [7] reported that plate fixation of femoral shaft fractures was associated with an average operative time of 104 minutes and an average fluoroscopy time of 109 seconds, both longer than in our study. However, Meccariello et al. [9] and Rollo et al. [21] reported shorter average operative times for plate fixation of femoral shaft fractures (62 min and 61 min). Regarding extramedullary fixation of the fractures extending in the subtrochanteric region, Yadav et al. [22] reported longer operation time (105 min) and fluoroscopy time (140 seconds) when using plates than in our study. El-Desouky et al. [19] compared conventional and biological plate fixation of subtrochanteric fractures and found that the operative time was longer (129 minutes *vs*. 92 minutes), while the fluoroscopy time was shorter (47 seconds *vs*. 80 seconds) when performing biological plate fixation [19].

In this regard, operative time in our study, for fractures extending into subtrochanteric region, corresponded more closely to conventional, while fluoroscopy time corresponded more closely to biological plate fixation. Erinc et al. [23] analyzed extramedullary plate fixation for supracondylar femoral fractures and reported longer operative time (126 minutes) compared to our findings for fractures extending into the supracondylar region. Comparative to the results in our study using SIF, the literature data listed above indicate both operative and fluoroscopy times tend to be longer when plate fixation is used for fractures extending into the subtrochanteric region, compared to fractures limited to the femoral shaft. It is noteworthy that similar trends have been reported for intramedullary fixation as well, with generally shorter operative times but longer fluoroscopy times than those observed in our study [5, 7, 9, 21, 22, 24, 25, 26].

Kelly et al. [27] found that the radiation dose received is significantly higher if the cumulative fluoroscopy time is less than 50 seconds. Considering this finding, the average results in our study suggest that the radiation dose in extramedullary fixation of femoral shaft fractures is generally lower (average fluoroscopy time was < 50 seconds) when using the SIF, in contrast to extramedullary fixation by plate reported in the literature, where the dose may be higher.

An earlier study by Mitkovic et al. [13] analyzed subtrochanteric fractures treated with SIF and reported that the average fluoroscopy time was almost the same (43 seconds) as in the present study on femoral shaft fractures extending into the subtrochanteric region, while the operative time was shorter (62 minutes). This difference in operative time may be explained by the fact that the subtrochanteric fracture study included various types of these fractures – not only femoral shaft fractures extending into the subtrochanteric region, but also those treated by SIF-troch implants shorter than 250 mm (subtrochanteric fractures that do not extend into the shaft region). The similarity between these studies regarding fluoroscopy time confirms that fluoroscopy is primarily used for lag screw placement when using an SIF-troch implant.

CONCLUSION

Operation time in extramedullary fixation of femoral shaft fractures using SIF is shortest when the fracture is confined to the shaft region only and longest when the fracture extends into the supracondylar region. Intraoperative fluoroscopy time is shortest for fractures limited to the femoral shaft, and longest for fractures extending into the subtrochanteric region. Considering the average fluoroscopy time, the use of SIF generally results in a low expected radiation dose, regardless of whether the femoral shaft fracture extends into an adjacent region or not.

ACKNOWLEDGEMENTS

This study is supported by the project "Virtual human osteoarticular system and its application in preclinical and clinical practice" [No. III41017 and 451-03-137/2025-03/200113], funded by the Ministry of Science, Technological Development and Innovation of Republic of Serbia.

Conflict of interest: None declared.

REFERENCES

1. Luthfi APWY, Hendarji A, Dalitan IM, Wedhanto S. Primary dynamic interlocking nail in femoral shaft fracture: A case series. Int J Surg Case Rep. 2023;105:108051. [DOI: 10.1016/j.ijscr.2023.108051] [PMID: 36996712]
2. Fu BS, Zheng ZH. Surgical treatments for femoral shaft fractures: A narrative review. Malays Orthop J. 2023;17(3):5–8. [DOI: 10.5704/MOJ.2311.002] [PMID: 38107348]
3. Salman LA, Al-Ani A, Radi MFA, Abudalou AF, Baroudi OM, Ajaj AA, et al. Open versus closed intramedullary nailing of femur shaft fractures in adults: a systematic review and meta-analysis. Int Orthop. 2023;47(12):3031–41. [DOI: 10.1007/s00264-023-05740-x] [PMID: 36864184]
4. Gupte D, Axelrod D, Worthy T, Woolnough T, Selznick A, Johal H. Management of femoral shaft fractures: The significance of traction or operative position. Cureus. 2023;15(1):e33776. [DOI: 10.7759/cureus.33776] [PMID: 36798626]
5. Ziegenhain F, Canal C, Halvachizadeh S, Mittlmeier A, Pape HC, Hierholzer C, et al. Outcome of femoral fractures treated with cerclages and intramedullary nailing. Eur J Trauma Emerg Surg. 2025;51(1):211. [DOI: 10.1007/s00068-025-02883-x] [PMID: 40392364]
6. Strait RT, Pankey C. Submuscular plating versus elastic intramedullary nailing in children with femoral shaft fracture; a systematic review and meta-analysis. J Clin Orthop Trauma. 2023;42:102203. [DOI: 10.1016/j.jcot.2023.102203] [PMID: 37529550]
7. Park KC, Oh CW, Byun YS, Oh JK, Lee HJ, Park KH, et al. Intramedullary nailing versus submuscular plating in adolescent femoral fracture. Injury. 2012;43(6):870–5. [DOI: 10.1016/j.injury.2011.10.032] [PMID: 22154047]
8. Hosseini H, Heydari S, Domari AA, Raesi R, Hushmandi K, Faryabi R, et al. Comparison of treatment results of femoral shaft fracture with two methods of intramedullary nail (IMN) and plate. BMC Surg. 2024;24(1):318. [DOI: 10.1186/s12893-024-02590-1] [PMID: 39425143]
9. Meccariello L, Bisaccia M, Ronga M, Falzarano G, Caraffa A, Rinonapoli G, et al. Locking retrograde nail, non-locking retrograde nail and plate fixation in the treatment of distal third femoral shaft fractures: radiographic, bone densitometry and clinical outcomes. J Orthop Traumatol. 2021;22(1):33. [DOI: 10.1186/s10195-021-00593-9] [PMID: 34350532]
10. Denisiuk M, Afsari A. Femoral Shaft Fractures. Treasure Island (FL): StatPearls Publishing; 2021. [PMID: 32310517]
11. Court-Brown C, McQueen M. M, Tornetta P. Trauma. Philadelphia: Lippincott Williams & Wilkins; 2006.
12. May C, Yen YM, Nasreddine AY, Hedequist D, Hresko MT, Heyworth BE. Complications of plate fixation of femoral shaft fractures in children and adolescents. J Child Orthop. 2013;7(3):235–43. [DOI: 10.1007/s11832-013-0496-5] [PMID: 24432082]
13. Mitkovic MM, Milenkovic S, Micic I, Kostic I, Stojiljkovic P, Mitkovic MB. Operation time and intraoperative fluoroscopy time in different internal fixation methods for subtrochanteric fractures treatment. Srp Arh Celok Lek. 2018;146(9–10):543–8. [DOI: 10.2298/SARH180220042M]
14. Zivanovic D, Radovanovic Z, Slavkovic A, Marjanovic Z. Internal fixator Mitkovic in the treatment of fractures of femoral shaft - a possible solution for fractures in heavier children and adolescents. Acta Chir Iug. 2015;62(1):45–7. [DOI: 10.2298/ACI1501045Z]

15. Mitkovic MB, Milenkovic S, Micic I, Mladenovic D, Mitkovic MM. Results of the femur fractures treated with the new selfdynamisable internal fixator (SIF). Eur J Trauma Emerg Surg. 2012;38(2):191–200. [DOI: 10.1007/s00068-011-0157-7] [PMID: 22611442]
16. Canciglieri OJ, Trajanovic MD, editors. Personalized orthopaedics. Cham: Springer; 2022. [DOI: 10.1007/978-3-030-98279-9]
17. Fu R, Liu Y, Song F, Fu J, Du T, Liu Y, et al. Effects of dynamization timing and degree on bone healing of different fracture types. J Orthop Res. 2023;41(11):2394–404. [DOI: 10.1002/jor.25583] [PMID: 37138390]
18. Fu R, Feng Y, Liu Y, Willie BM, Yang H. The combined effects of dynamization time and degree on bone healing. J Orthop Res. 2022;40(3):634–43. [DOI: 10.1002/jor.25060] [PMID: 33913530]
19. El-Desouky II, Mohamed MM, Kandil AE. Clinical outcome of conventional versus biological fixation of subtrochanteric fractures by proximal femoral locked plate. Injury. 2016;47(6):1309–17. [DOI: 10.1016/j.injury.2016.03.016] [PMID: 27015752]
20. Streubel PN, Gardner MJ, Morshed S, Collinge CA, Gallagher B, Ricci WM. Are extreme distal periprosthetic supracondylar fractures of the femur too distal to fix using a lateral locked plate? J Bone Joint Surg Br. 2010;92-B(4):527–34. [DOI: 10.1302/0301-620X.92B3.22996] [PMID: 20357329]
21. Rollo G, Bisaccia M, Rinonapoli G, Caraffa A, Pace V, Irimia JC, et al. Radiographic, bone densitometry and clinic outcomes assessments in femoral shaft fractures fixed by plating or locking retrograde nail. Med Arch. 2019;73(3):195–200. [DOI: 10.5455/medarh.2019.73.195-200] [PMID: 31404125]
22. Yadav S, Sinha S, Luther E, Arora NC, Prasad M, Varma R. Comparison of extramedullary and intramedullary devices for treatment of subtrochanteric femoral fractures at tertiary level center. Chin J Traumatol. 2014;17(3):141–5. [DOI: 10.3760/cma.j.issn.1008-1275.2014.03.004] [PMID: 24889976]
23. Erinc S, Cam N, Kanar M, Mustafa Ozdemir H. Comparison of two surgical techniques for periprosthetic supracondylar femoral fractures: Minimally invasive locking plate versus retrograde femoral nails. Sisli Etfal Hastan Tip Bul. 2021;55(4):477–85.
24. [DOI: 10.14744/SEMB.2021.34270] [PMID: 35317368]
25. Kang SJ, Bao FL, Huang DS, Jiang T, Hu YM, Li JM, et al. Percutaneous cerclage wiring combined with cephalomedullary nailing for irreducible subtrochanteric fractures. Orthop Surg. 2021;13(6):1899–911. [DOI: 10.1111/os.13144] [PMID: 34435729]
26. White M, Dejenie R, Tse S, Brooks B, Scott M, Chavez G, et al. Radiographic and clinical outcomes of traumatic subtrochanteric femur fracture fixation and reduction methods. Eur J Orthop Surg Traumatol. 2025;35(1):352. [DOI: 10.1007/s00590-025-04478-z] [PMID: 40826299]
27. Zhu Y, Lu B, Ouyang C, Gu S. Application of anterior minimally invasive clamping technique combined with lower extremity axial bone traction device in irreducible intertrochanteric fractures. Front Surg. 2025;12:1584651. [DOI: 10.3389/fsurg.2025.1584651] [PMID: 40969307]
28. Kelly GA, Rowan FE, Hurson C. Factors influencing radiation exposure during internal fixation of hip fractures. Eur J Orthop Surg Traumatol. 2017;27(5):637–41. [DOI: 10.1007/s00590-017-1951-7] [PMID: 28396949]

Утицај ширења прелома дијафизе бутне кости у суптрохантерну или супракондиларну регију на трајање операције и интраоперативне флуороскопије при динамичкој екстрамедуларној фиксацији

Милан М. Митковић[1,2], Саша С. Миленковић[1,2], Предраг М. Стојиљковић[1,2], Младен Ј. Стојановић[1], Никола Ј. Костић[1], Милош А. Нагорни[1]

[1]Универзитетски клинички центар Ниш, Клиника за ортопедију и трауматологију „Академик проф. др Милорад Митковић", Ниш, Србија;
[2]Универзитет у Нишу, Медицински факултет, Ниш, Србија

САЖЕТАК

Увод/Циљ Иако се преломи дијафизе бутне кости данас најчешће лече интрамедуларном фиксацијом, постоје ситуације у којима је пожељније извршити њихову екстрамедуларну фиксацију. Циљ овог рада било је испитивање утицаја ширења прелома дијафизе бутне кости у суседну анатомску регију на трајање операције и интраоперативне флуороскопије.

Методе У раду је анализирано 90 случајева код којих је прелом дијафизе бутне кости лечен самодинамизирајућим унутрашњим фиксатором (*SIF*). Испитаници су подељени у три групе – преломи дијафизе бутне кости који се шире проксимално (*SIF-troch*), преломи дијафизе бутне кости који се не шире ни проксимално ни дистално (*SIF-shaft*) и преломи дијафизе бутне кости који се шире дистално (*SIF-cond*).

Резултати Просечно трајање операције било је најкраће код прелома који се нису ширили у суседну регију (*SIF-shaft* група), а најдуже код прелома који су се ширили у супракондиларну регију (*SIF-cond* група). Просечно трајање флуороскопије било је најкраће у *SIF-shaft* групи, а најдуже код прелома који су се ширили у суптрохантерну регију (*SIF-troch* група). Сматра се да су на дужину операције у примењеним хируршким процедурама утицали техника контроле увођења клизних завртњева у проксимални део и закључавајућих завртњева у дистални део бутне кости, као и техника интраоперативног одржавања репозиције прелома у суптрохантерној и супракондиларној регији.

Закључак Ширење прелома дијафизе бутне кости у суптрохантерну или супракондиларну регију праћено је просечно дужим трајањем операције и флуороскопије. Уз пружање могућности динамизације у екстрамедуларној фиксацији, *SIF* пружа и релативно једноставну и брзо изводљиву технику фиксације прелома дијафизе бутне кости, нарочито када се прелом не шири у суседну анатомску регију.

Кључне речи: самодинамизирајући унутрашњи фиксатор; дијафиза; флуороскопија

DOI: https://doi.org/10.2298/SARH251002087M
UDC: 613-057.875

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Consumption of cigarettes, alcohol and energy drinks associated with academic performance and socioeconomic status of medical students

Jadranka M. Maksimović[1], Hristina D. Vlajinac[1], Isidora S. Vujčić[1], Slavica R. Maris[2], Katarina M. Maksimović[3], Tatjana B. Redžek Mudrinić[4,5], Ivana I. Kavečan[4,5]

[1]University of Belgrade, Faculty of Medicine, Institute of Epidemiology, Belgrade, Serbia;
[2]Institute of Public Health of Belgrade, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[4]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia;
[5]Institute for Children and Youth Health Care of Vojvodina, Novi Sad, Serbia

SUMMARY

Introduction/Objective University students from low-income families may face many challenges during their studies, which may affect their academic experience and outcomes. The aims of this study were to assess some lifestyle characteristics and academic performance of medical students and their relationship to family income.

Methods A cross-sectional study included 2551 undergraduate medical students at the Faculty of Medicine, in Belgrade, Serbia.

Results According to multivariate analysis, in comparison with students from families with income lower than two national average salaries, students with higher family income significantly more frequently finished gymnasium before the Faculty of Medicine, more frequently were in emotional relationships, were more frequently smokers and alcohol and energy drink consumers, and less frequently reported academic pressure.

Conclusion The present study emphasizes that socioeconomic status is not associated with the academic success of undergraduate medical students. Students from higher status feel less academic pressure, they more often consume cigarettes, alcohol, and energy drinks, and they are more likely to have an urban background and have highly educated parents who work as private company employees, which can be indicators of the specific economic climate in Serbia. Further studies are needed to strengthen evidence-based decision-making.

Keywords: alcohol consumption; cigarette smoking; energy drinks; epidemiology; medical students

INTRODUCTION

The Sustainable Development Goals, a set of 17 global goals adopted by all United Nations member states in October 2015, include reducing poverty in all its forms, reducing inequalities within and between countries, and ensuring inclusive and equitable quality education for all [1]. According to the World Bank, the Republic of Serbia is classified as an upper-middle-income country [2].

Income inequality in Serbia is one of the highest in Europe and higher than in any other member of the European Union. Socioeconomic and cultural backgrounds are related to students' lifestyle habits, perceived stress, and academic performance [3–7]. University students from low-income families may face many challenges during their studies, which may affect their academic experience and outcomes. Furthermore, the quality of life of medical students from the University of Tabriz in Iran has a positive correlation with family income [8]. Past research has shown that students from higher socioeconomic status (SES) are more likely to have higher-educated parents, which can be correlated with better parental support during the education process [9]. Additionally, family income can influence career aspirations. People with low SES more often have doubts about attending a faculty of medicine [10]. Also, a study conducted among Australian medical students found that students with very low and very high SES have less intention to work in low SES or medically inaccessible areas [11]. In addition, SES can be associated with students' mental health issues and substance use [12, 13, 14].

The aim of this study was to examine some characteristics, lifestyle, and academic performance of medical students in Belgrade, and their relationship to family income.

METHODS

Study participants

A cross-sectional study was conducted among undergraduate students at the Faculty of Medicine, University of Belgrade, one of the largest schools for the training of physicians in

Received • Примљено: October 2, 2025
Revised • Ревизија: November 16, 2025
Accepted • Прихваћено: November 17, 2025
Online first: November 26, 2025

Correspondence to:
Ivana KAVEČAN
Department of Pediatrics
Faculty of Medicine
University of Novi Sad
Hajduk Veljkova 3
21000 Novi Sad, Serbia
ivana.kavecan@mf.uns.ac.rs

Southeastern Europe, founded in 1920 in Belgrade, the capital of Serbia. It is part of the University of Belgrade, which was included among the top 500 universities according to the Academic Ranking of World Universities [15, 16]. The sample included 2551 medical students from all six study years with an overall response rate of 81.8%. The self-administered questionnaires were distributed during classes or practical sessions during clinical training. Students were asked to answer questions completely anonymously, after an explanation of the aim of the research. It took participants approximately 10 minutes to complete the questionnaire. We classified participants into two categories, depending on whether their family income was lower or higher than two average salaries in Serbia (88,000 RSD) or 2 × US$380 per month (adjusted net national income per capita) [17].

Data collection

The questionnaire

The questionnaire was constructed by the authors after a comprehensive literature search, and it contained questions about sociodemographic characteristics (gender, urban/rural background, family income, accommodation during the study, relationship status, parental education levels), lifestyle and personal characteristics (smoking, coffee, alcohol and energy drink consumption, average sleep duration, excessive spare time, academic achievement, and self-reported stress and pressure), and the Rosenberg self-esteem scale, a measurement test for self-esteem level widely used in behavioral and social research. The scale was designed by Morris Rosenberg, an American sociologist and social psychologist [18]. The questionnaire was composed of Likert-type scale questions.

The current smoker is defined as a person who has been smoking every day or several times a week, and who has smoked to date at least 100 cigarettes. Alcohol, coffee, and energy drink consumption, as well as self-reported stress, excessive spare time, and parental and academic pressure, were analyzed as yes/no variables.

Data analysis

Categorical variables (nominal and ordinal) were expressed as absolute and relative frequencies. For statistical analysis, we used SPSS version 17.0 for Windows. Univariate and multivariate logistic regression methods were applied to identify the independent variables associated with family income. Variables were selected for entry into the multivariate logistic regression model if they were significant in the univariate analysis with a $p \leq 0.1$. Results are expressed as an odds ratio (OR) with 95% confidence interval (CI) for the exponentiated regression coefficient (B).

Ethics: The study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Belgrade, Serbia (No. 1322/VII-45). Participants were recruited at the Faculty of Medicine, University of Belgrade.

RESULTS

The questionnaire was completed by 530 first-year students (response rate 93.1%), 470 second-year students (response rate 82.7%), 302 third-year students (response rate 61.7%), 459 fourth-year students (response rate 85.6%), 394 fifth-year students (response rate 75%), and 396 sixth-year students (response rate 91.9%). Some demographic and socioeconomic characteristics of the respondents are listed in Table 1. Female students made the majority of the sample (66.2%), as well as students who had an urban background (85.4%) and who had a grade point average (GPA) ≥ 8/10 (58.4%). Most of them came from families who had a monthly income lower than two national average salaries (61.8%) and whose parents worked as government employees (58.6%).

Table 1. Demographic and socioeconomic characteristics of medical students in Belgrade

Characteristics	Frequency	Percentage (%)
Gender		
Female	1690	66.2
Male	861	33.8
Class level		
First-year	530	20.8
Second-year	470	18.4
Third-year	302	11.8
Fourth-year	459	18
Fifth-year	394	15.4
Sixth-year	396	15.5
Original background		
Urban area	2179	85.4
Rural area	327	14.6
Average family income		
< Two national average salaries	1577	61.8
≥ Two national average salaries	944	38.2
The main source of family income		
Government employee	1494	58.6
Private company employee	828	32.5
Both	229	9

Table 2 displays differences between students from families who had a monthly income greater than the national average and students who came from a family with less income, which were analyzed by univariate logistic regression with $p \leq 0.1$ as the significance threshold. Students from higher-income families were significantly more likely to be male, to finish gymnasium before medical school, to have a GPA ≥ 8/10, to live in a private apartment during the academic term, and to be in a relationship. Furthermore, urban background, business or private company as major source of family income, and highly educated parents were significantly associated with descent from affluent families. Table 3 presents personal habits and perception of stress, pressure, and self-esteem. Being from higher-income families was significantly related to ≥ 6 hours average sleep duration, having too much spare time, and to consumption of cigarettes, alcohol, and energy drinks. Higher income was

Table 2. Characteristics of medical students in Belgrade according to socioeconomic status

Variable	Average monthly family income				p-value*
	< 88,000 RSD**		≥ 88,000 RSD†		
	Frequency	Percentage (%)	Frequency	Percentage (%)	
Gender					
Female	1080	68.5	610	62.6	0.002
Male	497	31.5	364	37.4	
High school					
Gymnasium	949	60.2	808	83.0	< 0.001
Medical high school	628	39.4	166	17.0	
Grade point average					
≥ 8/10	896	56.8	595	61.1	0.034
< 8/10	681	43.2	379	38.9	
Original background					
Urban area	1274	80.8	905	92.9	< 0.001
Rural area	65	19.2	69	7.1	
Accommodation during study					
Private apartment	1114	70.6	906	93.0	< 0.001
Dorm	463	29.4	68	7.0	
The main source of family income					
Government employee (at least one parent)	1470	93.2	852	87.5	< 0.001
Business / a private company employee	107	6.8	122	12.5	
Mother's education level					
Incomplete primary, primary and secondary	855	54.2	209	21.5	< 0.001
Higher	722	45.8	765	78.5	
Father's education level					
Incomplete primary, primary and secondary	780	49.5	182	18.7	< 0.002
Higher	797	50.5	792	81.3	
Relationship status					
In emotive relationship	693	43.9	497	51.0	< 0.001
Single	884	56.1	477	49.0	

RSD – Republic of Serbia dinars;
*according to univariate logistic regression analysis;
**< two national average salaries;
†≥ two national average salaries

Table 3. Lifestyle, perception of stress, pressure, and self-esteem among medical students in Belgrade, according to socioeconomic status

Variable	Average monthly family income				p-value*
	< 88,000 RSD (€715)**		≥ 88,000 RSD† (€715)		
	Frequency	Percentage (%)	Frequency	Percentage (%)	
Current smoker	282	17.9	271	27.8	< 0.001
Alcohol consumption	1020	64.7	754	77.4	< 0.001
Energy drinks consumption	586	37.2	396	40.7	0.078
Daily coffee consumption	884	56.1	572	58.7	0.186
Average sleep duration					
< 6 hours	686	43.5	374	38.4	0.011
≥ 6 hours	891	56.5	600	61.6	
Self-reported stress	1117	70.8	656	67.4	0.064
Self-reported excessive spare time	162	10.3	123	12.6	0.067
Self-reported pressure from parents	243	15.4	169	17.4	0.196
Self-reported academic pressure	1208	76.6	712	73.1	0.047
Low self-esteem	195	12.4	93	9.5	0.029

RSD – Republic of Serbia dinars;
*according to univariate logistic regression analysis;
**< two national average salaries;
†≥ two national average salaries

negatively associated with self-reported stress, academic pressure, and low self-esteem.

The results of a multivariate logistic regression analysis, which included significant variables from the univariate logistic regression, are shown in Table 4. According to multivariate analysis, in comparison with students from families with income lower than two national average salaries, students with higher family income significantly more frequently finished gymnasiums before medical school, had urban backgrounds, lived in a private apartment during the academic term, both parents worked as private company employees, and had highly educated parents.

Table 4. Factors associated with high family income of medical students in Belgrade (multivariate logistic regression analysis)

Characteristic (high/low family income)	Odds ratio	95% CI	p-value
Finished the gymnasium before the medical faculty	1.9	1.52–2.38	< 0.001
Urban background	1.65	1.21–2.23	0.001
Living in an apartment during faculty	4.22	3.17–5.62	< 0.001
Both parents work as private company employees	1.65	1.21–2.24	0.001
Highly educated mother	2.19	1.76–2.72	< 0.001
Highly educated father	2.29	1.83–2.86	< 0.001
Being in an emotive relationship	1.33	1.11–1.60	0.002
Being smoker	1.48	1.20–1.82	< 0.001
Alcohol consumption	1.48	1.19–1.85	< 0.001
Energy drinks consumption	1.27	1.05–1.54	0.013
≥ 6 hours average sleep duration	1.287	1.068–1.550	0.008
Academic pressure	0.74	0.92–0.60	0.005

They also were significantly more frequently in emotional relationships, were more frequently smokers and alcohol and energy drinks consumers, their average sleep duration was more frequently ≥ 6 hours, and they less frequently reported academic pressure.

DISCUSSION

The primary purpose of this study was to determine income-related characteristics among medical students in Belgrade. Consistent with the previous literature, our survey showed that students from lower SES are more likely to enroll in a vocational high school such as a medical high school, while higher-income students are more likely to enroll in a gymnasium – academic profile high school [19, 20].

We found that students from higher SES more often have an urban background and have highly educated parents and parents who work as private company employees. We believe that this background difference can align with the large regional disparities in Serbia in terms of socioeconomic conditions [21].

Univariate logistic regression analysis shows that students from higher SES have better academic performance, but the multivariate model didn't find this association, which was contrary to many previous studies [3, 4, 6]. This can be explained by the fact that we used only the GPA as an indicator of academic success. The GPA is a criterion for students' benefits in Serbia, such as a dormitory place or scholarships, which can motivate students from a family with lower income to learn more. Our study showed that students from lower SES are more likely to live in a dormitory (Table 4). We consider that this motivation for benefits can also be associated with increased pressure among students from a family with a lower income. According to the results of this survey, students from higher SES experience university life more comfortably. They more often answered that they did not feel academic pressure, that they slept ≥ 6 hours, and that they were in an emotional relationship (Table 4). Jury et al. [3] described in the literature review that lower-income students face psychological barriers, such as emotional distress and fear of failure. Past research also indicates that students from lower SES are at increased risk for depression and other mental health issues [12, 22, 23]. Counts et al. found a connection between low childhood SES and poor sleep quality of college students [24, 25].

In line with previous studies, our survey shows that higher income is associated with an increased rate of alcohol consumption among students and other young adults [26–29]. Higher income can also be related to other substance uses [14, 27]. The Balkan region, where Serbia is located, is characterized by the fact that people with a better financial situation are more likely to consume cigarettes [27]. Moreover, we have already described that students from high SES go through university with less academic pressure.

Magid et al. [30] consider smoking primarily as a social activity, and they also stated that stressful academic situations can distance students from situations where they can come in contact with cigarettes. This study may be subject to certain limitations. The survey was conducted at only one institution in the country and at only one faculty of the University of Belgrade. Also, students who have paused their studies or left the University were not included. Data about family income and other characteristics were based on self-reports, which can lead to recall bias. Another possible limitation could be information bias due to the classification of participants based on self-reports.

CONCLUSION

In summary, this study highlights that socioeconomic status is not associated with the academic success of undergraduate medical students, but students from higher status feel less academic pressure and feel more comfortable during university. They also more often consume cigarettes, alcohol, and energy drinks. Students from higher SES are more likely to have an urban background, to have highly educated parents and parents who work as private company employees, which can be indicators of the specific economic climate in Serbia. University authorities and policymakers should ensure a level playing field for all students, regardless of background. More studies need to be conducted in order to strengthen the implementation of evidence-based decisions.

Conflict of interest: None declared.

REFERENCES

1. United Nations. Sustainable Development Goals Report [Internet]. 2025 [cited 2025 Oct 15]. Available from: https://unstats.un.org/sdgs/files/report/2017/thesustainabledevelopmentgoalsreport2017.pdf
2. World Bank Country and Lending Groups [Internet]. 2019 [cited 2019 Jul 18]. Available from: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups
3. Jury M, Smeding A, Stephens M, Nelson E, Aelenei C, Darnon C. The Experience of Low-SES Students in Higher Education: Psychological Barriers to Success and Interventions to Reduce Social-Class Inequality. J Soc Issues. 2017;73(1):23–41. [DOI: 10.1111/josi.12202]
4. Murphy K, Kim T, Joy E, Shein B, Allan A. Social mobility through doctoral education: Exploring identity, classism, and belongingness. J Divers High Educ. 2025;18(1):16–24. [DOI: 10.1037/dhe0000477]
5. Maksimovic JM, Pavlovic AD, Vlajinac HD, Vujcic IS, Sipetic Grujicic SB, Maris SR, et al. Factors associated with smoking habits among undergraduate medical students: a cross-sectional study. J Subst Use. 2023;28(2):206–10. [DOI: 10.1080/14659891.2021.2019330]
6. Mahfouz A, Alhazmi F, Almatrafi K, Almehmadi S, Alharbi K, Qadi R, et al. The Influence of Lifestyle on Academic Performance Among Health Profession Students at Umm Al-Qura University. Cureus. 2024;16(3):e56759. [DOI: 10.7759/cureus.56759] [PMID: 38650788]
7. Lolokote S, Hidru TH, Li X. Do socio-cultural factors influence college students' self-rated health status and health-promoting lifestyles? A cross-sectional multicenter study in Dalian, China. BMC Public Health. 2017;17(1):478. [DOI: 10.1186/s12889-017-4411-8] [PMID: 28525995]
8. Ghassab-Abdollahi N, Shakouri SK, Aghdam AT, Farshbaf-Khalili A, Abdolalipour S, Farshbaf-Khalili A. Association of quality of life with physical activity, depression, and demographic characteristics and its predictors among medical students. J Educ Health Promot. 2020;9:147. [DOI: 10.4103/jehp.jehp_91_20] [PMID: 32766332]
9. Ely K, Lagasca G, Andersen S, Patel D, Simanton E. Medical Students' Socioeconomic Status and Academic Performance in Medical School. Cureus. 2023;15(6):e39875. [DOI: 10.7759/cureus.39875] [PMID: 37404444]
10. Gentry AN, Martin JP, Douglas KA. Social capital assessments in higher education: a systematic literature review. Front Educ. 2025;9:1498422. [DOI: 10.3389/feduc.2024.1498422]
11. Griffin B, Porfeli E, Hu W. Who do you think you are? Medical student socioeconomic status and intention to work in underserved areas. Adv Health Sci Educ Theory Pract. 2017;22(2):491–504. [DOI: 10.1007/s10459-016-9726-1] [PMID: 27812820]
12. Phomprasith S, Karawekpanyawong N, Pinyopornpanish K, Jiraporncharoen W, Maneeton B. Prevalence and Associated Factors of Depression in Medical Students in a Northern Thailand University: A Cross-Sectional Study. Healthcare. 2022;10(3):488. [DOI: 10.3390/healthcare10030488] [PMID: 35326966]
13. Sinval J, Oliveira P, Novais F, Almeida M, Telles-Correia D. Exploring the impact of depression, anxiety, stress, academic engagement, and dropout intention on medical students' academic performance: A prospective study. J Affect Disord. 2025;368:665–73. [DOI: 10.1016/j.jad.2024.09.116] [PMID: 39303881]
14. Domić A, Tahirović H, Nikić-Damjanovuć J, Čižek-Sajko M. The connection of the family's socioeconomic status and consumption of cigarettes, alcohol and marijuana in adolescents of the Brčko district of Bosnia and Herzegovina. Srp Arh Celok Lek. 2020;148(9–10):584–9. [DOI: 10.2298/SARH190717053D]
15. Shanghai Jiao Tong University. Academic Ranking of World Universities 2019 [Internet]. 2019 [cited 2019 Sep 22]. Available from: https://www.shanghairanking.com/rankings/arwu/2019
16. Shanghai Jiao Tong University. Academic Ranking of World Universities 2024 [Internet]. 2024 [cited 2024 Sep 22]. Available from: https://www.shanghairanking.com/rankings/arwu/2024
17. World Bank. Adjusted net national income per capita (current US$) [Internet]. [cited 2019 Sep 22]. Available from: https://data.worldbank.org/indicator/NY.ADJ.NNTY.PC.CD
18. Rosenberg M. Society and the adolescent self-image (Revised edition). Middletown, CT: Wesleyan University Press; 1989.
19. Giotakou A. Higher education and cultural capital: The role of parental education in Greek medical school choices. Int J Curr Educ Stud. 2024;3(2):37–50. [DOI: 10.46328/ijces.142]
20. Baucal A, Pavlović-Babić D. Kvalitet i pravednost obrazovanja u Srbiji: obrazovne šanse siromašnih analiza podataka PISA 2003 i 2006 [Internet]. Beograd: Ministarstvo prosvete Republike Srbije i Institut za psihologiju; 2009 [cited 2025]. Available from: http://www.inkluzija.gov.rs/wp-content/uploads/2010/03/Quality-and-Equity-ofEducation-in-Serbia-PISA-Assessment-2003-and-2006.pdf
21. Trifu S, Copcea B. Regional Disparities and Growth Dynamics in the Republic of Serbia. Economy Series/Analele Universităţii 'Constantin Brâncuşi' din Târgu-Jiu Seria Economie. 2015;(4):265–75.
22. Valladares-Garrido D, Quiroga-Castañeda PP, Berrios-Villegas I, Zila-Velasque JP, Anchay-Zuloeta C, Chumán-Sánchez M, et al. Depression, anxiety, and stress in medical students in Peru: a cross-sectional study. Front Psychiatry. 2023;14:1268872. [DOI: 10.3389/fpsyt.2023.1268872] [PMID: 38090694]
23. Aydın A, Akkuş Z. The Relationship Between University Students' Smoking Attitudes and Depression. Med J Bakirkoy. 2025;21(3):331–8. [DOI: 10.4274/BMJ.galenos.2025.2025.2-7]
24. Croock J, Mpinganjira G, Gathoo K, Bulmer R, Lautenberg S, Dlamini Q, et al. Probable depression and its correlates among undergraduate students in Johannesburg. Front Psychiatry. 2023;14:1018197. [DOI: 10.3389/fpsyt.2023.1018197] [PMID: 36873208]
25. Vasava PJ, Jasani PK, Sochaliya KM, Makwana MH, Kapadiya MR, Kadri SAS, et al. Assessing Sleep Quality and Influencing Factors Among Medical Undergraduates: A Cross-Sectional Study. Cureus. 2025;17(2):e78919. [DOI: 10.7759/cureus.78919] [PMID: 40091917]
26. Patrick ME, Wightman P, Schoeni RF, Schulenberg JE. Socioeconomic status and substance use among young adults: a comparison across constructs and drugs. J Stud Alcohol Drugs. 2012;73(5):772–82. [DOI: 10.15288/jsad.2012.73.772] [PMID: 22846241]
27. Vasiljevic D, Mihailovic N, Radovanovic S. Socioeconomic Patterns of Tobacco Use-An Example from the Balkans. Front Pharmacol. 2016;7:372. [DOI: 10.3389/fphar.2016.00372] [PMID: 27807419]
28. Ghaffari R, Osouli Tabrizi S, Abdolalipour S, Mirghafourvand M. Prevalence of cigarette smoking among iranian medical students and its relationship with mental health. Int J Health Promot Educ. 2022;61(1):1–12. [DOI: 10.1080/14635240.2022.2138719]
29. Taha H, Al-Maayeh A, Al Momani N, Natour LA, Abu Abboud S, AlRamahi AR, et al. Prevalence and Determinants of Tobacco Smoking Among University Students in Jordan: A Cross-Sectional Study. Tob Use Insights. 2025;18:1179173X251377625. [DOI: 10.1177/1179173X251377625] [PMID: 41018526]
30. Magid V, Colder R, Stroud R, Nichter M, Nichter M; TERN Members. Negative affect, stress, and smoking in college students: unique associations independent of alcohol and marijuana use. Addict Behav. 2009;34(11):973–5. [DOI: 10.1016/j.addbeh.2009.05.007] [PMID: 19523773]

Повезаност конзумирања цигарета, алкохола и енергетских пића са академским успехом и социоекономским статусом студената медицине

Јадранка М. Максимовић[1], Христина Д. Влајинац[1], Исидора С. Вујчић[1], Славица Р. Марис[2], Катарина М. Максимовић[3], Татјана Б. Рељек Мудринић[4,5], Ивана И. Кавечан[4,5]

[1]Универзитет у Београду, Медицински факултет, Институт за епидемиологију, Београд, Србија;
[2]Институт за јавно здравље Београда, Београд, Србија;
[3]Универзитет у Београду, Медицински факултет, Београд, Србија;
[5]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[4]Институт за здравствену заштиту деце и омладине Војводине, Нови Сад, Србија

САЖЕТАК

Увод/Циљ Студенти из породица са ниским приходима могу се суочити са многим изазовима током студија, што може утицати на њихово академско искуство и резултате. Циљеви ове студије били су да се процене одређене карактеристике начина живота и академски успех студената медицине и њихов однос са породичним приходима.

Методе Студија пресека обухватила је 2551 студента основних студија медицине на Медицинском факултету у Београду, Србија.

Резултати Према мултиваријантној анализи, у поређењу са студентима из породица са приходима нижим од две просечне националне плате, студенти са вишим породичним приходима значајно чешће завршавају гимназију пре медицинског факултета, чешће су у емотивним везама, чешће су пушачи и конзументи алкохола и енергетских пића, а ређе пријављују академски притисак.

Закључак Ова студија наглашава да социоекономски статус није повезан са академским успехом студената медицине. Студенти са вишим статусом осећају мањи академски притисак, чешће конзумирају цигарете, алкохол и енергетска пића, вероватније је да су градског порекла и да имају високообразоване родитеље који су запослени у приватним компанијама, што могу бити показатељи специфичне економске климе у Србији. Потребно је спровести додатне студије како би се ојачала примена одлука заснованих на доказима.

Кључне речи: конзумирање алкохола; пушење цигарета; енергетска пића; епидемиологија; студенти медицине

DOI: https://doi.org/10.2298/SARH250816085P
UDC: 616.13-007.64

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Ruptured aneurysm of the superficial femoral artery

Miroslava Popović[1], Ivana Mitrović Tanić[1], Marko Šurlan[1], Ljiljana Milić[1,2]

[1]Zvezdara University Clinical Hospital Center, Nikola Spasić Clinic for Surgery, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction Aneurysm rupture is rare, most often affecting the distal third of the superficial femoral artery (SFA). An isolated aneurysm of the SFA is even rarer, occurring in only 1% of all femoral artery aneurysms and in 0.5% of all peripheral artery aneurysms. The aim of this report is to describe the diagnosis and surgical treatment of a rare case of a ruptured true aneurysm of the SFA.

Case report We present an 80-year-old man admitted due to sudden pain in his right leg. Physical examination and radiological imaging confirmed a ruptured isolated SFA aneurysm. SFA–SFA bypass surgery was performed in the distal femoral region of the right leg three months after the first symptoms occurred. An SFA–SFA bypass using an 8 mm Dacron graft was performed, and the patient achieved full recovery with palpable pedal pulses at the one-month follow-up. Two months after hospital discharge, the patient died from acute myocardial infarction, making further follow-up impossible.

Conclusion This report demonstrates that even three months after SFA aneurysm rupture, complete recovery can be achieved with an appropriate surgical technique.

Keywords: superficial femoral artery; ruptured aneurysm; true femoral aneurysm; rupture

INTRODUCTION

Superficial femoral artery (SFA) aneurysm is more common in men than in women, with an incidence of about 85%. Factors contributing to the development of a true SFA aneurysm include smoking, age, and cardiovascular disease [1].

An isolated aneurysm of the SFA is extremely rare, occurring in only 1% of all femoral artery aneurysms and 0.5% of all peripheral artery aneurysms [2]. Aneurysm rupture is uncommon, most frequently affecting the distal third of the SFA [3]. The complications most often associated with SFA aneurysms include rupture, distal embolization, and thrombosis [4].

The objective of this report is to describe the diagnosis and surgical treatment of a rare case of a ruptured SFA aneurysm.

CASE REPORT

An 80-year-old man complained of pain in the lower third of the right thigh. Five days after the onset of pain, the symptoms worsened, and swelling of the right thigh appeared. Two days later, swelling and hematoma around the right upper knee were confirmed at the local hospital.

Upon hospital admission, his international normalized ratio was 5.2, hemoglobin 80 g/dl, and he received two units of blood. He denied any allergies to medication or food. He was a non-smoker and did not consume alcohol. His medical history included chronic obstructive pulmonary disease, type 2 diabetes, and hypothyroidism. In the year 1992, he had a myocardial infarction without subsequent revascularization.

Three months later, he was admitted to our institution, pulmonology department, due to shortness of breath, pain in the right upper knee, and severe calf spasms. He was afebrile, with blood pressure of 105/60 mmHg and a heart rate of 70 beats per minute. D-dimer level was 15.2 mg/L fibrinogen equivalent unit, and chest multidetector computed tomographic angiography revealed bilateral peripheral microembolism.

Two days later, major swelling appeared in the lower right thigh (Figure 1a). The patient was unable to stretch his leg, and the knee joint was locally warm at the site of the swelling. A Doppler scan of the lower extremities showed a hypoechogenic mass measuring approximately 40 × 48 cm, with monophasic flow in the popliteal and posterior tibial arteries. The popliteal vein of right leg was partially non-compressible, with suspicion of a fresh thrombus. No other deep vein thrombosis was detected.

Multidetector computed tomographic angiography of the right leg showed rupture of the posterior wall in the distal third of the SFA, with contrast extravasation and a hematoma over 300 mm in length, extending below the knee and compressing the surrounding structures (Figure 1b). A large saccular aneurysm of the internal iliac artery, 51 mm in diameter, was also diagnosed. The patient was transferred to the vascular surgery department for further treatment.

Emergency surgery revealed a large amount of coagulum and blood extending from the

Received • Примљено: August 16, 2025
Revised • Ревизија: November 13, 2025
Accepted • Прихваћено: November 14, 2025
Online first: November 19, 2025

Correspondence to:
Ljiljana MILIĆ
Pančićeva 4
11000 Belgrade
Serbia
ljilja.milic@gmail.com

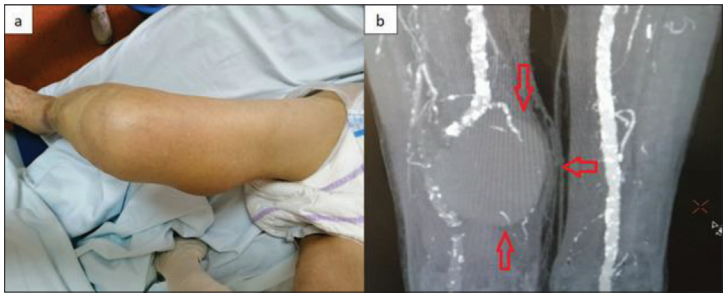


Figure 1. a – Clinical finding of rupture the right superficial femoral artery aneurism rupture; b – computed tomography angiography of the lower limb shows aneurism of superficial femoral artery with compression of neighboring structures (labeled by red arrows)

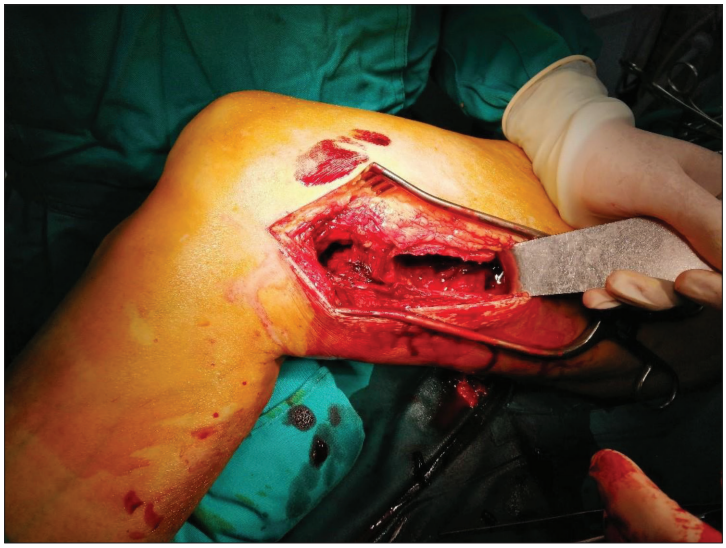


Figure 2. Intraoperative finding of ruptured aneurysm of the superficial femoral artery

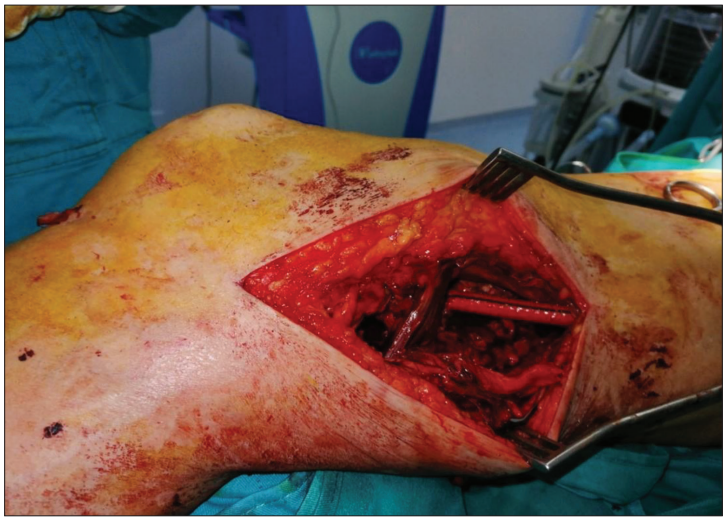


Figure 3. Superficial femoral artery bypass – reconstruction using Dacron 8 mm graft

distal part of the right thigh to the lower limb, with a ruptured aneurysm of the SFA (Figure 2). An SFA–SFA bypass was performed using an 8 mm Dacron graft (Figure 3). During the operation, the patient received one unit of erythrocytes, one unit of fresh frozen plasma, and three units of albumin. Postoperative laboratory findings were within normal limits.

On the first postoperative day, palpable dorsalis pedis pulse was noted, with preserved motility and sensation. On the 20th postoperative day, the patient was discharged home in good general condition and fully recovered.

At the one-month follow-up, the patient had palpable pedal pulses and no complaints. During the preparation for elective surgical treatment of the iliac artery aneurysm, the patient developed an acute myocardial infarction and died two months after hospital discharge, which made further follow-up impossible.

Ethics: The patient agreed to have his photos and medical information published in the journal. He was aware that his name will not be revealed and that every effort will be made to maintain his anonymity.

DISCUSSION

Atherosclerotic aneurysms of the SFA are very rare, occurring as isolated lesions in 15–25% of cases. They most often occur in the lower third of the SFA, typically as focal arterial involvement, while diffuse or long-segment involvement is extremely uncommon [3, 5]. In our patient, the aneurysm was located in the lower third of the SFA but involved a 7 cm segment, which is rare.

SFA aneurysms are frequently associated with aortic and iliac artery aneurysms (69%), and with popliteal or common femoral artery aneurysms in 54% of cases [6]. Our patient had a saccular aneurysm of the internal iliac artery measuring 51 mm in diameter. Since the patient did not present with acute symptoms related to this aneurysm, urgent intervention was not indicated. Elective surgical treatment was planned following full recovery and appropriate preparation for the procedure.

At the time of diagnosis, approximately 35% of patients with isolated SFA aneurysms are symptomatic, which is significantly higher than for common femoral artery aneurysms, where only about 7% of patients are symptomatic [7]. This difference may be explained by the anatomical localization and the deep position of the artery, which make early detection and elective surgery more difficult. Clinical recognition is also challenging in thin patients because of the deep muscular position of the vessel [6].

Rupture is the most common presentation of SFA aneurysms, occurring in 26–34% of cases, which is much higher than in popliteal artery aneurysms, where rupture is the initial symptom in only about 3% of cases [6, 8]. The patient in this report presented initially with rupture, consistent with previous studies.

Surgical treatment is indicated in symptomatic patients with ruptured SFA aneurysms or ischemic symptoms. In asymptomatic cases, there is still no consensus on the appropriate timing for intervention, though some authors recommend surgery when the aneurysm diameter exceeds 20–25 mm [3, 9].

Both open and endovascular repair are viable treatment options. Open repair provides favorable short- and long-term outcomes with excellent limb salvage rates [10]. The most common procedure is aneurysmectomy with prosthetic graft interposition [3]. In our case, aneurysmectomy was performed three months after rupture, with reconstruction using an 8 mm Dacron graft. The great saphenous vein was not used due to varicosities, because both great saphenous veins were varicose and unsuitable for reconstruction in our patient, we decided to implant a Dacron graft. The choice of procedure was based on clinical experience and literature-reported efficacy for aneurysms of similar etiology.

SFA rupture presents differently from other peripheral arterial ruptures, as it may enlarge significantly before detection. Early recognition and prompt surgical intervention offer the best chance of survival and full recovery. Although SFA aneurysms are rare, any hematoma in the upper leg especially near the course of the femoral artery should be considered a potential aneurysm until proven otherwise.

This case demonstrates that even three months after rupture of an SFA aneurysm, complete recovery can be achieved with an appropriate surgical approach.

ACKNOWLEDGMENT

This work was presented as a poster presentation at the XXIX World Congress of the International Union of Angiology and the I Virtual World Congress of the International Union of Angiology in June 2020.

Conflict of interest: None declared.

REFERENCES

1. Peterson CI, Royalty J. Expanding Saccular Aneurysm of the Superficial Femoral Artery Managed With Endovascular Grafting: A Case Report. Cureus. 2025;17(1):e78013. [DOI: 10.7759/cureus.78013] [PMID: 40013184]
2. Christoforou P, Kounnos C, Kapoulas K, Bekos C. Ruptured true aneurysm of the superficial femoral artery leading to Behçet disease diagnosis. J Vasc Surg Cases Innov Tech. 2022;8(3):473–6. [DOI: 10.1016/j.jvscit.2022.06.002] [PMID: 36035229]
3. Perini P, Jean-Baptiste E, Vezzosi M, Raynier JL, Mottini F, Batt M, et al. Surgical management of isolated superficial femoral artery degenerative aneurysms. J Vasc Surg. 2014;59(1):152–8. [DOI: 10.1016/j.jvs.2013.07.011] [PMID: 24199768]
4. Corriere MA, Guzman RJ. True and false aneurysms of the femoral artery. Semin Vasc Surg. 2005;18(4):216–23. [DOI: 10.1053/j.semvascsurg.2005.09.008] [PMID: 16360579]
5. Papageorgopoulou CP, Nikolakopoulos KM. Endovascular treatment of a ruptured true superficial femoral artery aneurysm. Radiol Case Rep. 2021;16(7):1685–7. [DOI: 10.1016/j.radcr.2021.04.031]. Erratum in: Radiol Case Rep. 2023;18(3):1383–4. [DOI: 10.1016/j.radcr.2022.10.047] [PMID: 34007384]
6. Jarrett F, Makaroun MS, Rhee RY, Bertges DJ. Superficial femoral artery aneurysms: an unusual entity? J Vasc Surg. 2002;36(3):571–4. [DOI: 10.1067/mva.2002.125841] [PMID: 12218983]
7. Miyamotto M, Biscardi JMDS, Trentin CD, Machado RM, Angelo BZ, de Andrade DC, et al. Spontaneous rupture of superficial femoral artery aneurysm: case report. J Vasc Bras. 2019;18:e20180113. [DOI: 10.1590/1677-5449.011318] [PMID: 31236105]
8. Piffaretti G, Mariscalco G, Tozzi M, Rivolta N, Annoni M, Castelli P. Twenty-year experience of femoral artery aneurysms. J Vasc Surg. 2011;53(5):1230–6. [DOI: 10.1016/j.jvs.2010.10.130] [PMID: 21215583]
9. Traina L, Zenunaj G, Bisogno F, Scian S, Acciarri P, Medini C, et al. Incidence, Diagnosis, Treatment, and Outcomes of True Superficial Artery Aneurysms. Ann Vasc Surg. 2021;76:565–72. [DOI: 10.1016/j.avsg.2021.04.044] [PMID: 34175418]
10. Bashar K, Ali SS, Garnham A. Review of the management of isolated superficial femoral artery aneurysms. Indian J Vasc Endovasc Surg. 2021;8:19–24. [DOI: 10.4103/ijves.ijves_28_20]

Руптура анеуризме површинске бутне артерије

Мирослава Поповић[1], Ивана Митровић Танић[1], Марко Шурлан[1], Љиљана Милић[1,2]

[1]Универзитетски клиничко-болнички центар „Звездара“, Клиника за хирургију „Никола Спасић“, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод Руптура анеуризме површинске феморалне артерије представља изузетно редак клинички ентитет, са учесталошћу мањом од 1% свих периферних анеуризми, укључујући и псеудоанеуризме, односно свега 0,5% када се ради о правим периферним анеуризмама. Циљ овог рада је приказ дијагностике и хируршког лечења ретке руптуре праве анеуризме површинске феморалне артерије.

Приказ болесника Приказујемо болесника старости 80 година, примљеног због изненадног бола у десној нози. Клиничким прегледом и радиолошком дијагностиком потврђена је руптура изоловане анеуризме површинске феморалне артерије. Три месеца након појаве првих симптома изведен је *SFA–SFA* бајпас са дакронским графтом пречника 8 *mm*, а болесник се потпуно опоравио, са опипљивим пулсовима на стопалима на контролном прегледу после месец дана. Два месеца након отпуста из болнице болесник је преминуо од акутног инфаркта миокарда, што је онемогућило даље праћење.

Закључак Овај приказ потврђује да је и три месеца после руптуре анеуризме површинске феморалне артерије могуће постићи потпуни терапијски успех уз примену адекватне хируршке методе.

Кључне речи: површинска феморална артерија; руптура анеуризме; права феморална анеуризма; руптура

DOI: https://doi.org/10.2298/SARH250718088P
UDC: 616.61-089.84

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

The role of nonadherence in donor-specific antibodies formation and their effects on kidney transplant function

Lada Petrović[1], Igor Mitić[1], Dejan Ćelić[2], Milica Popović[2], Gordana Stražmešter-Majstorović[2]

[1]University of Novi Sad, Faculty of Medicine, University Clinical Center of Vojvodina, Center for Transplantation of Organs, Cells and Tissues, Novi Sad, Serbia;
[2]University of Novi Sad, Faculty of Medicine, University Clinical Center of Vojvodina, Clinic of Nephrology and Clinical Immunology, Novi Sad, Serbia

SUMMARY

Introduction Antibody-mediated rejection is one of the leading causes of graft loss after kidney transplant. Donor-specific antibodies (DSAs) are recognized as biomarkers of transplant rejection. The aim of this study was to describe the association between nonadherence and DSA formation.

Case outline A 21-year-old patient underwent a living-related donor kidney transplant procedure in October 2017. The donor had the same blood type as the patient with one mismatch at the HLA-B and HLA-DR loci. The presence of pre-transplant human leukocyte antigen donor-specific antibodies (HLA-DSA) was not confirmed. The postoperative course was uneventful. Three months post-transplant, low tacrolimus levels and consequent increase of serum creatinine were evident. Five months post-transplant, the occurrence of HLA-DSA was confirmed along with *de novo* donor-specific anti-HLA-DQB1*06:04, mean fluorescence intensity (MFI) was 20,725. Acute antibody-mediated rejection of kidney transplant was diagnosed, and the following treatment was applied: corticosteroid pulses, immunoglobulins, and plasmapheresis. Stable graft function persisted over the following one-year period, but over time, low tacrolimus levels, increase in serum creatinine, and proteinuria reappeared. Heteroanamnestic data indicated irregular taking of immunosuppressive drugs and an inadequate hygiene-dietary regimen. Repeated anti-HLA-DQB1*06:04 testing revealed MFI of 5933. Graft biopsy demonstrated elements of chronic active antibody-mediated rejection, acute T-cell-mediated rejection, interstitial fibrosis, and tubular atrophy. Despite repeated anti-rejection therapy, total graft loss occurred.

Conclusion Nonadherence to recommended immunosuppressive regimen brought about the *de novo* HLA-DSA formation as well as production of antibody-mediated and T-cell-mediated rejection, and consequent total loss of kidney transplant function.

Keywords: kidney transplant; nonadherence; donor-specific antibodies

INTRODUCTION

Antibody-mediated rejection has been recognized as the leading cause of graft dysfunction and graft loss after kidney transplant. Antibodies against the human leukocyte antigen play a major role in this process, thus making it a critical barrier for solid organ transplantation. Precise and timely detection of human leukocyte antigen (HLA) donor-specific antibodies (DSAs) is vital for evaluating humoral immune status of patients pre- and posttransplantation. According to the occurrence time and type of immune response, HLA-DSAs are distributed into three groups: 1. HLA-DSAs identified before kidney transplant (preformed HLA-DSAs) can cause early rejection, such as hyperacute rejection, accelerated acute rejection, early acute antibody-mediated rejection, and graft loss; 2. *de novo* HLA-DSAs developed after transplant are associated with late acute antibody-mediated rejection, chronic antibody-mediated rejection, and transplant glomerulopathy; 3. "benign" HLA-DSAs are not considered clinically relevant because they are not associated with antibody-mediated rejection and graft loss [1].

The technology of screening antibodies has advanced from the complement-dependent cytotoxicity assay, enzyme-linked immunosorbent assay, to multiplexed particle-based flow cytometry (Luminex) – a qualitative microbead-based immunoassay for the detection of both class I and II IgG anti-HLA antibodies. Single antigen beads are used to characterize the preformed HLA-DSAs before transplant as well as any *de novo* development of HLA-DSAs after transplant [2, 3].

Current transplant practices recommend against offering a kidney from the donor expressing an unacceptable HLA antigen (positive virtual crossmatch). Only the patients whose HLA antibodies are not donor-directed will appear on the match run (negative virtual crossmatch).

The development of de novo HLA-DSAs after kidney transplant was reported in 13–30% of previously nonsensitized patients. The risk factors for *de novo* HLA-DSAs include the following: 1) high HLA mismatches (especially DQ

Received • Примљено: July 18, 2025
Revised • Ревизија: November 8, 2025
Accepted • Прихваћено: November 21, 2025
Online first: November 26, 2025

Correspondence to:
Lada PETROVIĆ
University Clinical Center of Vojvodina
Center for Transplantation of Organs, Cells and Tissues
Faculty of Medicine
University of Novi Sad
Hajduk Veljkova 1
21000 Novi Sad, Serbia
lada.petrovic@mf.uns.ac.rs

mismatches), 2) inadequate immunosuppression and nonadherence, and 3) graft inflammation, which can increase graft immunogenicity. *De novo* HLA-DSAs are predominantly directed to donor HLA class II mismatches and usually occur during the first year of kidney transplant but can appear at any time, even several years later. HLA-DSA binding to antigen expressed on allograft endothelial cells can activate the classic complement pathway, a key pathological process of acute antibody-mediated rejection phenotypes [1]. Some HLA-DSAs can cause graft damage through antibody-dependent cellular cytotoxicity and induce subclinical and chronic antibody-mediated rejection phenotypes. Furthermore, HLA-DSAs can cause graft injury by direct activation of endothelial proliferation and consequent development of transplant glomerulopathy and vasculopathy.

According to the World Health Organization (WHO), adherence to long-term therapy is defined as the degree to which the person's behavior corresponds with the agreed recommendations from a responsible health care provider (physician, nurse) with regard to the type and dosage of drugs, dietary regimen, daily habits, and work–life balance. Nonadherence is quite common after kidney transplant, occurring in about 22% of patients (reported prevalence rates range 8–55% in some transplant centers) [4, 5, 6]. Intentional nonadherence is manifested by deliberate modification of treatment recommendations by the patient, such as irregular or improper taking of prescribed medication (e.g. omission on weekends or holidays, skipping the dose, taking lower or higher doses than prescribed, changing dosing intervals, consuming drugs at an improper time of the day, taking the wrong drug, complete discontinuance of the therapy). Nonadherence also includes nonattendance at scheduled control examinations, avoiding or rejecting laboratory appointments. Risk factors for noncompliant behavior of the patient after kidney transplant can be attributed to the patient themself, transplant center, or therapy regimen. Patient-related factors can pertain to age, sex, renal transplantation without a previous period on dialysis, education level, socioeconomic factors, taking psychoactive substances, and history of previous nonadherence with other therapeutic procedures. Factors associated with the transplant center include inadequate pre- and posttransplant education, poor communication and lack of confidence in the transplant team, and period after the transplantation procedure. Potential lack of cooperation between patient and health care provider may be attributed to the therapeutic regimens implicating a wide range of diverse drugs, adverse effects of drugs, as well as high medication costs.

It is important to differentiate adherence from compliance. According to WHO, adherence requires the patient's commitment and active participation in the treatment, relying on good communication between the patient and health care provider as the prerequisite for a successful clinical course. Contrary to that, compliance represents a passive following of medical advice, where the patient is regarded as an object and solely a recipient of care [4].

Besides other factors associated with graft loss, such as glomerulonephritis, polyoma virus nephropathy, medical/surgical conditions, antibody-mediated rejection is responsible for graft loss in more than 50% of cases (64% of cases). Within this sample population, a high percentage (47%) was associated with the *de novo* formation of DSAs due to nonadherence [6]. Accordingly, *de novo* DSAs are associated with a significant reduction in 10-year graft survival *vs.* in the no *de novo* DSA group [7].

The aim of this study was to describe the association between nonadherence and *de novo* DSA formation with consequent rejection and permanent loss of kidney transplant function.

CASE REPORT

The patient was subjected to chronic hemodialysis in December 2016, with chronic tubulointerstitial nephritis as the most probable underlying cause of end-stage renal disease (kidney biopsy was not performed since the disease had been diagnosed at a highly advanced stage). In October 2017, the 21-year-old patient underwent kidney transplant from a living-related donor with a matching blood type. HLA typing revealed one mismatch at the HLA-B and one in HLA-DR loci (MM 2/6) with a negative final crossmatch with fresh serum from the recipient and lymphocytes from the donor (CDC). Induction therapy included a monoclonal antibody [IL-2 receptor blocker (basiliximab (Simulect, Novartis Pharma AG, Basel, Switzerland), 20 mg on days 1 and 4)] and methylprednisolone (750 mg; 10 mg/kg body weight). Tacrolimus, mycophenolate mofetil, and prednisone were used as immunosuppressive maintenance therapy. Serum samples from the recipient were analyzed for class I and class II IgG HLA antibodies using a qualitative microbead-based immunoassay based on a Luminex platform. The presence of donor-specific class I and class II IgG HLA antibodies was confirmed neither six months nor one month before (prospective) as well as 15 days after the transplant procedure. Also, complement-dependent cytotoxicity (CDC) assay performed one month before transplantation did not reveal the presence of class I and class II HLA-DSAs.

Immediate postoperative course at the Department for Transplant Surgery was uneventful, without complications and with a gradual decrease of serum creatinine levels (value at discharge from hospital: creatinine = 110 μmol/L), satisfactory diuresis, while ultrasonographic examination revealed normal graft morphology and patency of vascular structures. Low levels of tacrolimus (2.3 ng/mL) were observed at the regular outpatient control examination performed three months posttransplant (January 2018) followed by gradual increase of serum creatinine levels, which reached twice its initial value after five months (in March 2018). The patient was hospitalized and underwent additional examination to identify the reasons for graft function impairment. The following results were obtained: negative urine and blood BK virus DNA PCR, negative cytomegalovirus DNA PCR, and hemolytic uremic syndrome was excluded. Qualitative detection of IgG antibodies in recipient's serum revealed the presence of class I and class II

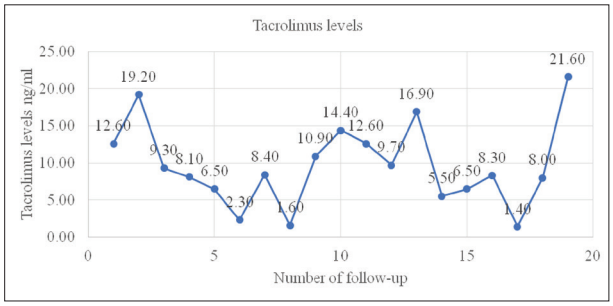


Figure 1. Tacrolimus levels during follow-up

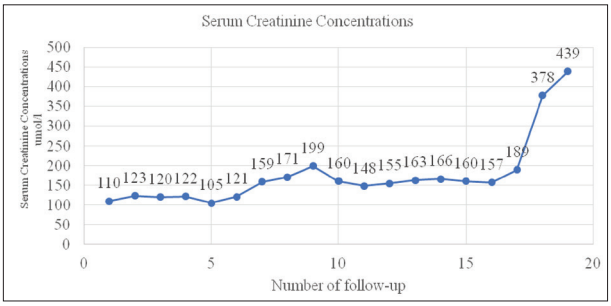


Figure 2. Serum creatinine concentrations during follow-up

HLA-DSAs, with very high anti-HLA-DQB1*06 antibody titer and mean fluorescence intensity (MFI) values being anti-HLA-DQB106:01 MFI = 21,446, 06:02 MFI = 19,870, 06:03 MFI = 20,507, 06:04 MFI = 20,725. It was confirmed that anti-HLA-DQB106:04 antibody was a donor-specific *de novo* formed antibody (supplementary high-resolution HLA typing confirmed that the donor was a DQB106:04 carrier). Acute antibody-mediated rejection of kidney transplant was diagnosed and treated with corticosteroid pulses, immunoglobulins (total 50 g) combined alternately with five plasmapheresis sessions. The treatment resulted in gradual normalization of serum creatinine levels (maximum creatinine level was 226 μmol/L, creatinine level at the end of the therapy was 133 μmol/L). Monitoring of serum tacrolimus levels and dosage adjustment was performed. The dose of antihypertensive drugs was increased to stabilize arterial hypertension. Stable graft function persisted over the following one-year period, that is, until January 2019, when low tacrolimus levels (1.4 ng/mL), increase in serum creatinine, and proteinuria were detected again. Heteroanamnestic data indicated an irregular taking of immunosuppressive drugs as well as an inadequate hygiene-dietary regimen during Christmas and New Year holidays. Repeated HLA-DSAs testing revealed the presence of class I and class II anti-HLA-DQB1 IgG antibodies, yet with significantly lower MFI values as compared to those recorded in March 2018 (anti-HLA-DQB1*06:04 MFI = 5933). Percutaneous graft biopsy was performed. Histopathological analysis revealed morphologic changes in all nephron components, C4d-positive staining in < 10% of peritubular capillaries, chronic active antibody-mediated rejection (2b), acute T-cell-mediated rejection (Banff grade IA), interstitial fibrosis, and tubular atrophy (I) according to the Banff classification. Corticosteroid pulses, immunoglobulins (0.5 g/kg body mass), and five plasmapheresis sessions were prescribed. The treatment did not result in the desired therapeutic response; thus, total graft loss occurred (Figures 1 and 2).

Ethics: Before the start of the study, approval was granted by the Ethics Committee of the University Clinical Center of Vojvodina, Novi Sad, Serbia (No.: 00-281). Written informed consent was obtained from the patient to publish this case report.

DISCUSSION

As far back as some 30 years ago, the age of the patient was considered to play an important role in nonadherence after renal transplant. Relative risk for adherence to medical recommendations in patients over 50 and younger than 20 was 1.564 and 0.800 (95% CI), respectively [8]. Moreover, kidney transplant from a living-related donor (as was the case in this article) is frequently reported as the reason for nonadherence, as compared with cadaveric transplantation. nonadherence occurs most commonly and is particularly pronounced during holiday seasons [9, 10, 11].

Nonadherence leads to suboptimal immunosuppression and consequent alloimmune activation and graft loss. Posttransplantation nonadherence to prescribed immunosuppressive regimen has been identified as an independent risk factor for unfavorable clinical course and a cause of 36% of kidney transplant losses [9]. Considering its importance and vital effects on immunosuppressive regimen, nonadherence is suggested to be regarded as the "fifth vital sign," which should be timely identified through regular monitoring of immunosuppressive drug levels (e.g. tacrolimus) and *de novo* formed DSAs. Problem identification and development of a personalized action plan with specific solutions (simplified medication regimen, education, and psychological behavioral support) are pivotal [12, 13, 14].

In the presented case, the unfavorable clinical course is to be attributed to nonadherence to recommended immunosuppressive regimen. Nonadherence has provoked suboptimal immunosuppression with consequent *de novo* formation of HLA-DSA and, most likely, primary antibody-mediated rejection. Continuous nonadherence further resulted in acute T-cell-mediated rejection with elements of chronic active antibody-mediated rejection and complete loss of function of the transplanted kidney.

Conflict of interest: None declared.

REFERENCES

1. Zhang R. Donor-specific antibodies in kidney transplant recipients. Clin J Am Soc Nephrol. 2018;13(1):182–92. [DOI: 10.2215/CJN.00700117] [PMID: 28446536]
2. Van den Broek DAJ, Meziyerh S, Budde K, Lefaucheur C, Cozzi E, Bertrand D, et al. The clinical utility of post-transplant monitoring of donor-specific antibodies in stable renal transplant recipients: a consensus report with guideline statements for clinical practice. Transpl Int. 2023;36:11321. [DOI: 10.3389/ti.2023.11321] [PMID: 37560072]
3. López Del Moral C, Wu K, Naik M, Osmanodja B, Akifova A, Lachmann N, et al. The natural history of de novo donor-specific HLA antibodies after kidney transplantation. Front Med (Lausanne). 2022;9:943502. [DOI: 10.3389/fmed.2022.943502] [PMID: 36186822]
4. World Health Organization. Adherence to long-term therapies: evidence for action. Geneva: World Health Organization; 2003.
5. Gandolfini I, Palmisano A, Fiaccadori E, Cravedi P, Maggiore U. Detecting, preventing and treating non-adherence to immunosuppression after kidney transplantation. Clin Kidney J. 2022;15(7):1253–74. [DOI: 10.1093/ckj/sfac017] [PMID: 35756738]
6. Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. Am J Transplant. 2012;12(2):388–99. [DOI: 10.1111/j.1600-6143.2011.03840.x] [PMID: 22081892]
7. Cherukuri A, Mehta R, Sharma A, Sood P, Zeevi A, Tevar AD, et al. Post-transplant donor specific antibody is associated with poor kidney transplant outcomes only when combined with both T-cell-mediated rejection and non-adherence. Kidney Int. 2019;96(1):202–13. [DOI: 10.1016/j.kint.2019.01.033] [PMID: 31029504]
8. Zachciał J, Uchmanowicz I, Czapla M, Krajewska M, Banasik M. Association between psychosocial and age-related factors and adherence to immunosuppressive therapies after renal transplantation. J Clin Med. 2022;11(9):2386. [DOI: 10.3390/jcm11092386] [PMID: 35566514]
9. Mahmood K, Allen J, Varley R, Vrijens B, Huuskes BM, Woywodt A. 10 tips to improve adherence to immunosuppressive medication after kidney transplantation. Clin Kidney J. 2025;18(6):sfaf164. [DOI: 10.1093/ckj/sfaf164] [PMID: 40521289]
10. Corr M, Walker A, Maxwell AP, McKay GJ. Non-adherence to immunosuppressive medications in kidney transplant recipients: a systematic scoping review. Transplant Rev (Orlando). 2025;39(1):100900. [DOI: 10.1016/j.trre.2024.100900] [PMID: 39642406]
11. Carratalá-Munuera C, Cortés-Castell E, Márquez-Contreras E, Castellano JM, Perez-Paramo M, López-Pineda A, et al. Barriers and solutions to improve therapeutic adherence from the perspective of primary care and hospital-based physicians. Patient Prefer Adherence. 2022;16:697–707. [DOI: 10.2147/PPA.S319084] [PMID: 35300358]
12. Park Y, Lee H, Eum SH, Kim HD, Ko EJ, Yang CW, et al. Intrapatient variability in tacrolimus trough levels over 2 years affects long-term allograft outcomes of kidney transplantation. Front Immunol. 2021;12:746013. [DOI: 10.3389/fimmu.2021.746013] [PMID: 34659243]
13. Han A, Jo AJ, Kwon H, Kim YH, Lee J, Huh KH, et al. Optimum tacrolimus trough levels for enhanced graft survival and safety in kidney transplantation: a retrospective multicenter real-world evidence study. Int J Surg. 2024;110(10):6711–22. [DOI: 10.1097/JS9.0000000000001800] [PMID: 38884261]
14. Neuberger JM, Bechstein WO, Kuypers DRJ, Burra P, Citterio F, De Geest S, et al. Practical recommendations for long-term management of modifiable risks in kidney and liver transplant recipients: a guidance report and clinical checklist by the Consensus on Managing Modifiable Risk in Transplantation (COMMIT) Group. Transplantation. 2017;101(4S Suppl 2):S1–S56. [DOI: 10.1097/TP.0000000000001651] [PMID: 28328734]

Улога непридржавања терапије у настанку антитела специфичних за донора и њихов утицај на функцију трансплантираног бубрега

Лада Петровић[1], Игор Митић[1], Дејан Ћелић[2], Милица Поповић[2], Гордана Стражмештер-Мајсторовић[2]

[1]Универзитет у Новом Саду, Медицински факултет, Универзитетски клинички центар Војводине, Центар за трансплантацију органа, ћелија и ткива, Нови Сад, Србија;

[2]Универзитет у Новом Саду, Медицински факултет, Универзитетски клинички центар Војводине, Клиника за нефрологију и клиничку имунологију, Нови Сад, Србија

САЖЕТАК

Увод Антителима посредовано одбацивање један је од водећих узрока губитка графта након трансплантације бубрега. Антитела специфична за донора (*DSA*) представљају један од биомаркера овог процеса, а циљ рада је био да прикаже улогу неадхеренције у њиховом настанку.

Приказ болесника Болеснику старом 21 годину, у октобру 2017. године, урађена је трансплантација бубрега од живог, сродног даваоца исте крвне групе, са једним неподударањем у *HLA-B* и *HLA-DR* локусу. Пре трансплантације није доказано присуство анти-*HLA* антитела специфичних за донора (*HLA-DSA*). Постоперациони ток је протекао без компликација. Три месеца након трансплантације запажен је низак ниво такролимуса, после чега је уследио пораст концентрације серумског креатинина. Пет месеци након трансплантације доказано је присуство *HLA-DSA*, са новостворeним антителом специфичним за донора, анти-*HLA-DQB*1*06:04, средњег интензитета флуоресценције (*mean fluorescence intensity – MFI*) од 20.725. Закључено је да се ради о акутном, антителима посредованом одбацивању трансплантираног бубрега, те је примењена следећа терапија: пулсна кортикостероидна терапија, имуноглобулини, плазмафереза. Стабилна функција графта одржана је наредних годину дана, када су се поново регистровали низак ниво такролимуса, пораст серумског креатинина и појава протеинурије. Хетероанамнестички су добијени подаци о нередовном узимању имуносупресивних лекова и неадекватном хигијенско-дијететском режиму живота. Поновљена анализа антитела анти-*HLA-DQB*1*06:04 показала је вредност *MFI* од 5933. Биопсијом графта нађени су елементи хроничног активног антителима посредованог одбацивања, акутног Т-ћелијама посредованог одбацивања, интерстицијалне фиброзе и тубуларне атрофије (класификација по Банфу). И поред поновљене терапије против одбацивања, дошло је до потпуног губитка функције графта.

Закључак Непридржавање препорученог имуносупресивног режима довело је до настанка *de novo HLA-DSA*, као и до развоја антителима и Т-ћелијама посредованог одбацивања, са последичним потпуним губитком функције трансплантираног бубрега.

Кључне речи: трансплантација бубрега; неадхеренција; антитела специфична за донора

DOI: https://doi.org/10.2298/SARH250610095B
UDC: 618.14-006.6

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Insulin resistance as a risk factor for endometrial cancer – a case report of fertility-sparing treatment of early-stage endometrial cancer

Aleksandra Beleslin[1], Olga Mihaljević[1], Branislav Milošević[1,2], Igor Pilić[1,2], Katarina Stefanović[1,2]

[1]University Clinical Centre of Serbia, Clinic for Gynaecology and Obstetrics, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction Endometrial cancer is the most common gynecologic malignancy, and up to a quarter of cases are diagnosed in patients under the age of 45. Important risk factors that create a hyperestrogenic environment are obesity, polycystic ovary syndrome, insulin resistance, and type 2 diabetes mellitus. The standard treatment is classic hysterectomy with bilateral salpingo-oophorectomy; however, this treatment leads to loss of fertility, which poses an issue for younger patients who have not completed childbearing. Therefore, in certain cases, hormonal therapy could be used for early-stage endometrial adenocarcinoma to preserve fertility.

Case outline A 32-year-old female patient with insulin resistance presented with an ultrasonographic finding of an endometrial polyp, and after hysteroscopy and thorough evaluation, a well-differentiated adenocarcinoma of the endometrium (G1), FIGO stage IA, was verified. A conservative treatment was carried out with a levonorgestrel intrauterine device and a gonadotropin-releasing hormone (GnRH) analog for six months. After six months of therapy, there were no signs of malignant cells, and she conceived naturally. Eventually, the patient delivered a healthy baby.

Conclusion Insulin resistance is a potentially modifiable risk factor and thus important in cases of fertility preservation treatment. Management could reduce cancer risk and improve reproductive outcomes. Further studies are needed to better understand the impact of insulin resistance treatment on the success of fertility- sparing management and the rate of recurrence.

Keywords: endometrial cancer; oncofertility; fertility-sparing treatment of endometrial cancer; insulin resistance

INTRODUCTION

Endometrial cancer (EC) has an incidence of 4.3% and is the most common malignancy of the genital tract among Caucasians [1]. Available data suggest that up to a quarter of cases are diagnosed in patients under the age of 45 who have not yet completed childbearing [2]. In these cases, the diagnosis is often made incidentally after hysteroscopy or curettage of the uterine cavity, typically performed as part of an infertility evaluation, due to ultrasound findings of an endometrial polyp or irregular bleeding. Although most histopathological types of EC are considered hormone-sensitive, lifestyle and environmental factors have a significant impact on the development of cancer [3]. Known risk factors for EC include age, race, early menarche, late menopause, nulliparity, and conditions that create a hyperestrogenic environment, such as obesity, polycystic ovary syndrome, insulin resistance (IR), type 2 diabetes mellitus, and metabolic syndrome [4]. Metabolic disorders characterized by hyperinsulinemia can impact carcinogenesis through various molecular mechanisms [3]. IR is a fundamental component of metabolic syndrome, and many studies have linked IR to cancer [5]. Genetic predisposition, such as Lynch syndrome and BRCA mutations, is also a significant nonmodifiable risk factor [6, 7].

The importance of discussing treatment options for EC among premenopausal women is significant. The standard treatment is hysterectomy with bilateral salpingo-oophorectomy [8]. However, this treatment results in the loss of reproductive function, which poses an issue for younger patients who have not completed childbearing and wish to become pregnant. Thus, in certain cases, hormonal therapy could be used as a treatment option for early-stage adenocarcinoma of the endometrium to preserve fertility [9]. This treatment option is also important in terms of quality of life because five-year survival rate of stage I EC is 85% [10].

According to the guidelines of the leading European societies for gynecologic oncology [the European Society of Gynecological Oncology (ESGO)], radiotherapy and oncology [the European Society for Radiotherapy and Oncology (ESTRO)], and pathology [the European Society of Pathology (ESP)], a conservative treatment approach could be taken for patients under 45 years old with well-differentiated early-stage endometrial adenocarcinoma [9]. Hormonal therapy may include oral progestins, GnRH analogs, and an intrauterine device with levonorgestrel. In case of complete

Received • Примљено: June 10, 2025
Revised • Ревизија: November 21, 2025
Accepted • Прихваћено: December 4, 2025
Online first: December 11, 2025

Correspondence to:
Aleksandra BELESLIN
Clinic for Gynaecology and Obstetrics
University Clinical Centre of Serbia
Koste Todorovića 26
Belgrade 11000
Serbia
aleksandrabeleslin@gmail.com

response, pregnancy is recommended. After successful pregnancy and completing childbearing, definitive surgery (standard hysterectomy) is advised, as the recurrence rate can be as high as 25% [11].

Since EC is strongly associated with modifiable risk factors such as insulin resistance, timely recognition and adequate treatment are important. It could be substantial for EC prevention, the success of fertility- sparing treatment, and lowering recurrence risk. Therefore, the aim of this case is to emphasize the significance of modifiable risk factors in a cancer patient.

CASE REPORT

A 32-year-old female patient, G0P0, presented with an ultrasonographic finding of an endometrial polyp during a regular checkup, with regular menstrual cycles and no irregular intermenstrual bleeding. Apart from insulin resistance calculated by the homeostatic model assessment index, there was no other comorbidity. The patient was taking only metformin. Body mass index was normal (20 kg/m^2). Family history was unremarkable. As a gold standard for endometrial polyp evaluation, hysteroscopy was performed. Well-differentiated adenocarcinoma of the endometrium (G1) was verified after hysteroscopy, polypectomy, and uterine curettage. The next step was to determine the clinical stage according to the International Federation of Gynecology and Obstetrics (FIGO) classification [12]. An MRI of the abdomen and pelvis was performed to rule out myometrial invasion, adnexal involvement, and concomitant ovarian tumor. According to pelvic MRI, the endometrial lining was nonhomogeneous, with a hypovascular 3-mm lesion within the endometrium without myometrial invasion; the endometrial–myometrial junction was intact.

To initiate conservative treatment, assessment by two expert pathologists is required to confirm the diagnosis, which was also done, confirming the diagnosis of endometrioid adenocarcinoma (G1) of the endometrium. Standard evaluation for patients preparing for fertility- sparing treatment includes a chest radiograph, hormonal and thyroid status assessment, Pap smear for cervical cancer screening, and breast ultrasound. After complete evaluation, an early stage of well-differentiated adenocarcinoma (FIGO stage IA) was diagnosed. Considering the type and stage of EC, young age, nulliparity, and a strong desire to preserve fertility, the clinical board approved conservative treatment that involved insertion of a levonorgestrel intrauterine device along with a GnRH analog for six months. The ultrasound examination after three months of therapy was unremarkable, and no side effects were reported. Menstrual bleeding ceased after three months of therapy. Intrauterine device removal and a follow-up hysteroscopy with curettage of the uterine cavity were performed after six months. Histopathological examination showed no atypia or malignant cells. After two negative biopsies six months apart and three months after the last hysteroscopy, the patient conceived spontaneously. She had an uncomplicated pregnancy and delivered a healthy male child weighing 4500 g via elective cesarean section. Postpartum hysteroscopy and curettage revealed no signs of malignancy.

Ethics: According to the journal's position on issues involving ethical publication, written consent for publication of this article has been obtained from the patient.

DISCUSSION

The case of a young patient with insulin resistance as a risk factor for endometrial cancer, who was successfully treated conservatively with hormone therapy, is presented.

There are two types of endometrial cancer that differ in their pathogenesis, aggressiveness, and prognosis. The far more common type is type I, which is found in almost 90% of cases [8]. It is considered an estrogen-dependent, well-differentiated cancer and is associated with insulin resistance, obesity, and type 2 diabetes mellitus [8, 10]. Type I occurs more frequently before menopause and during early menopause and has a favorable prognosis [8]. In contrast, type II is estrogen-independent, less differentiated, occurs in older patients, and carries a higher risk of rapid progression and an unfavorable outcome [3, 13].

The case presented is a type I, well-differentiated carcinoma that occurred in a young patient with no symptoms. The incidence of this malignancy is increasing in women younger than 50 years old [14]. This trend could be linked to today's sedentary lifestyle and the higher incidence of risk factors among the younger population, such as obesity, insulin resistance, and type 2 diabetes mellitus [15].

The only risk factor noted in this patient was insulin resistance. The influence of insulin resistance on the development of malignancy can be explained by metabolic dysregulation involving inflammatory cytokines, growth factors, various enzymes, and free fatty acids [16]. Elevated insulin levels, chronic inflammation, and hyperactivation of growth pathways are associated with the development and progression of cancer [17]. An important metabolic pathway activated by insulin and insulin-like growth factor 1 (IGF-1) is the PI3K/AKT/mTOR pathway, leading to cell proliferation, invasion, and metastasis [16]. This pathway is also crucial for understanding the effect of treatment with metformin, which is the drug of choice [18]. Metformin leads to the suppression of the mTOR signaling pathway, reducing the concentration of insulin and IGF-1, thus suppressing protein translation and cell proliferation [19]. Although previous studies' results are controversial regarding the reduction of cancer incidence in patients with diabetes treated with metformin, it is still important to recognize risk factors [16, 20]. Timely recognition of risk factors such as insulin resistance, diabetes, and obesity and their treatment as part of conservative treatment for early-stage endometrial cancer is valuable because it reduces the effect of insulin and IGF-1 on the endometrium [21]. Insulin is thought to influence estrogen receptor expression, thus affecting endometrial proliferation and potential

carcinogenesis [22]. This influence may contribute to a better endometrial response to the local action of the levonorgestrel intrauterine device.

A levonorgestrel-releasing intrauterine device, alone or in combination with oral progestins or GnRH analogs, is a recommended option for conservative treatment of endometrial adenocarcinoma [9]. The latest guideline from ESGO advises a levonorgestrel intrauterine device and/or oral progestins as first-line treatment [9]. GnRH analogs are an alternative therapy with protective effects on ovarian reserve, contributing to improved pregnancy rates, and many studies reported satisfactory results with GnRH analogs [9]. Standard protocol in our institution for conservative treatment of early-stage endometrial cancer consisted of a levonorgestrel intrauterine device with GnRH analogs for six months and was introduced over a decade ago [23].

Although no statistical significance was observed in studies examining the effect of insulin resistance on mortality after hysterectomy in endometrial cancer, the effect of insulin resistance on the outcome of conservative treatment could be more significant because the uterus remains, and the endometrium could still undergo malignant transformation [20]. This is supported by the results of the study by Li et al. [21], who showed that the time to relapse in patients with endometrial cancer treated conservatively is significantly shorter in those with insulin resistance compared to those without. Also, considering the normal values of the body mass index in our patient, the influence of obesity and peripheral conversion of estrogen on the endometrium and tumor formation is ruled out.

Furthermore, recognizing risk factors is important because they affect fertility, fertilization, and could complicate pregnancy, and the aim of conservative treatment of endometrial cancer is to achieve a successful pregnancy [9]. Insulin resistance is linked to recurrent miscarriages, gestational diabetes, and gestational hypertension [19, 24, 25]. This impacts the fetus, leading to macrosomia and the need for operative delivery, which carries its own risks [25]. The best way to prevent these complications is to establish proper glycemic control before conception [19]. Despite our patient's normal oral glucose tolerance test during pregnancy, the newborn weighed 4500 g, exceeding the 90th percentile for that gestational age [26].

Nevertheless, in terms of the generally high survival rate for early-stage endometrial cancer, the aforementioned risk factors are associated with lifelong cardiovascular morbidity and mortality [10]. This should be noted especially in cases of definitive radical treatment where the protective estrogen effect is lost.

Insulin resistance is recognized as a significant and potentially modifiable risk factor for endometrial cancer. Its role could be particularly important in cases of fertility preservation treatment. Early identification and management of metabolic abnormalities could reduce cancer risk and improve reproductive outcomes. A multidisciplinary approach, including an endocrinologist, is essential to optimize cancer treatment and reproductive potential. Further studies are needed to better understand the impact of insulin resistance treatment on the success of fertility-sparing management and the rate of recurrence.

Conflict of interest: None declared.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. [DOI: 10.3322/caac.21660] [PMID: 33538338]
2. Contreras NA, Sabadell J, Verdaguer P, Julià C, Fernández-Montolí ME. Fertility-Sparing Approaches in Atypical Endometrial Hyperplasia and Endometrial Cancer Patients: Current Evidence and Future Directions. Int J Mol Sci. 2022;23(5):2531. [DOI: 10.3390/ijms23052531] [PMID: 35269674]
3. Sidorkiewicz I, Jóźwik M, Niemira M, Krętowski A. Insulin Resistance and Endometrial Cancer: Emerging Role for microRNA. Cancers (Basel). 2020;12(9):2559. [DOI: 10.3390/cancers12092559] [PMID: 32911852]
4. Raglan O, Kalliala I, Markozannes G, Cividini S, Gunter MJ, Nautiyal J, et al. Risk factors for endometrial cancer: An umbrella review of the literature. Int J Cancer. 2019;145(7):1719–30. [DOI: 10.1002/ijc.31961] [PMID: 30387875]
5. Chiefari E, Mirabelli M, La Vignera S, Tanyolaç S, Foti DP, Aversa A, et al. Insulin Resistance and Cancer: In Search for a Causal Link. Int J Mol Sci. 2021;22(20):11137. [DOI: 10.3390/ijms222011137] [PMID: 34681797]
6. Ryan NAJ, McMahon R, Tobi S, Snowsill T, Esquibel S, Wallace AJ, et al. The proportion of endometrial tumours associated with Lynch syndrome (PETALS): A prospective cross-sectional study. PLoS Med. 2020;17(9):e1003263. [DOI: 10.1371/journal.pmed.1003263] [PMID: 32941469]
7. Kitson SJ, Bafligil C, Ryan NAJ, Lalloo F, Woodward ER, Clayton RD, et al. BRCA1 and BRCA2 pathogenic variant carriers and endometrial cancer risk: A cohort study. Eur J Cancer. 2020;136:169–75. [DOI: 10.1016/j.ejca.2020.05.030] [PMID: 32698099]
8. Crosbie EJ, Kitson SJ, McAlpine JN, Mukhopadhyay A, Powell ME, Singh N. Endometrial cancer. Lancet. 2022;399(10333):1412–28. [DOI: 10.1016/S0140-6736(22)00323-3] [PMID: 35397864]
9. Rodolakis A, Scambia G, Planchamp F, Acien M, Di Spiezio Sardo A, Farrugia M, et al. ESGO/ESHRE/ESGE Guidelines for the fertility-sparing treatment of patients with endometrial carcinoma. Hum Reprod Open. 2023;2023(1):hoac057. [DOI: 10.1093/hropen/hoac057] [PMID: 36756380]
10. Rundle-Thiele D, Shrestha S, Janda M. Prevention of endometrial cancer through lifestyle interventions: A systematic review and synthesis. Gynecol Oncol Rep. 2021;39:100900. [DOI: 10.1016/j.gore.2021.100900] [PMID: 35531361]
11. Qin Y, Yu Z, Yang J, Cao D, Yu M, Wang Y, et al. Oral Progestin Treatment for Early-Stage Endometrial Cancer: A Systematic Review and Meta-analysis. Int J Gynecol Cancer. 2016;26(6):1081–91. [DOI: 10.1097/IGC.0000000000000723] [PMID: 27177279]
12. Berek JS, Matias-Guiu X, Creutzberg C, Fotopoulou C, Gaffney D, Kehoe S, et al; Endometrial Cancer Staging Subcommittee, FIGO Women's Cancer Committee. FIGO staging of endometrial cancer: 2023. J Gynecol Oncol. 2023;34(5):e85. [DOI: 10.3802/jgo.2023.34.e85] [PMID: 37593813]
13. Setiawan VW, Yang HP, Pike MC, McCann SE, Yu H, Xiang YB, et al. Type I and II endometrial cancers: have they different risk factors? J Clin Oncol. 2013;31(20):2607–18. [DOI: 10.1200/JCO.2012.48.2596] [PMID: 23733771]
14. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74(1):12–49. Erratum in: CA Cancer J Clin. 2024;74(2):203. [DOI: 10.3322/caac.21820] [PMID: 38230766]
15. Pillon NJ, Loos RJF, Marshall SM, Zierath JR. Metabolic consequences of obesity and type 2 diabetes: Balancing genes and environment for personalized care. Cell. 2021;184(6):1530–44. [DOI: 10.1016/j.cell.2021.02.012] [PMID: 33675692]

16. Kim DS, Scherer PE. Obesity, Diabetes, and Increased Cancer Progression. Diabetes Metab J. 2021;45(6):799–812. [DOI: 10.4093/dmj.2021.0077] [PMID: 34847640]
17. Orgel E, Mittelman SD. The links between insulin resistance, diabetes, and cancer. Curr Diab Rep. 2013;13(2):213–22. [DOI: 10.1007/s11892-012-0356-6] [PMID: 23271574]
18. Barczyński B, Frąszczak K, Kotarski J. Perspectives of metformin use in endometrial cancer and other gynaecological malignancies. J Drug Target. 2022;30(4):359–67. [DOI: 10.1080/1061186X.2021.2005072] [PMID: 34753372]
19. Lei Y, Yi Y, Liu Y, Liu X, Keller ET, Qian CN, et al. Metformin targets multiple signaling pathways in cancer. Chin J Cancer. 2017;36(1):17. [DOI: 10.1186/s40880-017-0184-9] [PMID: 28126011]
20. Morielli AR, Kokts-Porietis RL, Benham JL, McNeil J, Cook LS, Courneya KS, et al. Associations of insulin resistance and inflammatory biomarkers with endometrial cancer survival: The Alberta endometrial cancer cohort study. Cancer Med. 2022;11(7):1701–11. [DOI: 10.1002/cam4.4584] [PMID: 35174651]
21. Li X, Fan Y, Wang J, Zhou R, Tian L, Wang Y, et al. Insulin Resistance and Metabolic Syndrome Increase the Risk of Relapse For Fertility Preserving Treatment in Atypical Endometrial Hyperplasia and Early Endometrial Cancer Patients. Front Oncol. 2021;11:744689. [DOI: 10.3389/fonc.2021.744689] [PMID: 34917501]
22. Tian W, Teng F, Zhao J, Gao J, Gao C, Sun D, et al. Estrogen and insulin synergistically promote type 1 endometrial cancer progression. Cancer Biol Ther. 2017;18(12):1000–10. [DOI: 10.1080/15384047.2017.1394547] [PMID: 29172956]
23. Stefanovic K, Dotlic J, Pilic I, Milosevic B, Mihaljevic O, Beleslin A, et al. A Decade of Experience in Diagnostic and Conservative Treatment of Endometrial Malignancy-Oncologic and Obstetrical Outcomes from a Referral Oncofertility Center. Diagnostics (Basel). 2025;15(11):1388. [DOI: 10.3390/diagnostics15111388] [PMID: 40506958]
24. Lei R, Chen S, Li W. Advances in the study of the correlation between insulin resistance and infertility. Front Endocrinol (Lausanne). 2024;15:1288326. [DOI: 10.3389/fendo.2024.1288326] [PMID: 38348417]
25. Gou BH, Guan HM, Bi YX, Ding BJ. Gestational diabetes: weight gain during pregnancy and its relationship to pregnancy outcomes. Chin Med J (Engl). 2019;132(2):154–60. [DOI: 10.1097/CM9.0000000000000036] [PMID: 30614859]
26. Hadlock FP, Harrist RB, Martinez-Poyer J. In utero analysis of fetal growth: a sonographic weight standard. Radiology. 1991;181(1):129–33. [DOI: 10.1148/radiology.181.1.1887021] [PMID: 1887021]

Инсулинска резистенција као фактор ризика за карцином ендометријума – приказ случаја конзервативног лечења раног стадијума карцинома ендометријума

Александра Белеслин[1], Олга Михаљевић[1], Бранислав Милошевић[1,2], Игор Пилић[1,2], Катарина Стефановић[1,2]

[1]Универзитетски клинички центар Србије, Клиника за гинекологију и акушерство, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод Карцином ендометријума најчешћи је гинеколошки малигнитет и скоро четвртина случајева се дијагностикује код болесница млађих од 45 година. Важни фактори ризика који стварају хиперестрогену средину су гојазност, синдром полицистичних јајника, инсулинска резистенција и дијабетес мелитус типа 2. Стандардни вид лечења је класична хистеректомија са билатералном аднексектомијом, али овај третман доводи до губитка фертилитета, што представља проблем за млађе болеснице које још нису остариле репродуктивну функцију. У одређеним случајевима могуће је спровести хормонску терапију као вид конзервативног лечења у циљу очувања фертилитета.

Приказ болесника Болесници старој 32 године, са инсулинском резистенцијом као фактором ризика, урађена је хистероскопска полипектомија због ултразвучног налаза ендометријалног полипа. Након хистопатолошке верификације и евалуације дијагностикован је добро диференован аденокарцином ендометријума, стадијум IA. Спроведен је конзервативни третман применом интраутериног улошка са левоноргестрелом и *GnRH* аналога током шест месеци. С обзиром на то да је после шестомесечне терапије урађена контролна хистероскопија и да хистопатолошки налаз није указао на присуство малигнитета, саветована је трудноћа. Болесница је спонтано затруднела, успешно је изнела терминску трудноћу и родила здраво мушко дете.

Закључак Инсулинска резистенција је фактор ризика на који можемо да утичемо и тако смањимо ризик од настанка малигнитета, али и побољшамо репродуктивни исход. Њена улога је посебно важна у конзервативном лечењу карцинома ендометријума и зато су потребна додатна истраживања како би се боље разумео утицај лечења инсулинске резистенције на успех конзервативног лечења, али и на појаву рецидива.

Кључне речи: карцином ендометријума; онкофертилитет; поштедно лечење карцинома ендометријума; инсулинска резистенција

DOI: https://doi.org/10.2298/SARH250706091B
UDC: 617.713-007.23

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Mystery corneal opacity ring

Marija Božić, Tanja Kalezić, Vesna Marić, Bojana Dačić Krnjaja

University of Belgrade, Faculty of Medicine, University Clinical Center of Serbia, University Eye Hospital, Belgrade, Serbia

SUMMARY

Introduction We present a case of an incidental finding of unusual ring-like bilateral corneal opacities in otherwise clear corneas in a person with no other ophthalmological disease except cataract. Clinical findings, laser scanning *in vivo* confocal microscopy and corneal topography findings do not correspond to any described corneal degeneration, so the aim of this presentation is to consult with fellow ophthalmologists regarding the origin and significance of the described changes.

Case outline "Mystery corneal ring" is found incidentally as a bilateral, asymptomatic, almost perfectly round in shape, white and situated adjacent to limbus. The appropriate diagnostics performed did not give an answer as to the origin of these symmetrical opacites.

Conclusion We present previously undescribed symmetrical bilateral corneal opacities, in search of an answer as to whether any of our fellow ophthalmologists have had similar cases in their practice or know what type of disorder it is.

Keywords: cornea; opacity; dystrophy

INTRODUCTION

Congenital anomalies of the cornea are changes of a wide spectrum and do not all have to lead to a decrease in visual acuity or other symptoms. Corneal findings in those cases are most often bilateral, although they can be asymmetric and are very well classified and described. However, occasionally in clinical practice we encounter a finding on the cornea that does not correspond to any entity described so far. The aim of this case report is to share our findings with a wider ophthalmological audience and to obtain the opinion of our colleagues regarding the origin of the described changes.

CASE REPORT

A 75-year-old woman presented for cataract surgery. Best corrected visual acuity (Snellen eye chart) was 0.4 in her right and 0.6 in her left eye. The corneas were otherwise normal, with single regular, ring shaped, opacification approximately 3 mm from the limbus. The epithelium overlying the hazed part of the cornea was regular. Cortical and nuclear opacities were noted in right lens. Intraocular pressures (Goldmann applanation tonometry) were 14 and 16 mmHg. Cup to disc ratio was 0.3/0.3 in both of her eyes. Central corneal thickness was 525 μm and 524 μm. Corneal sensations were normal in both eyes. Corneal opacity did not affect visual acuity.

We did laser scanning *in vivo* confocal microscopy and corneal topography that reviled sharply demarcated single corneal ring, with smooth surface, and no deposits or pigmentations, and band like condensations in corneal stroma (Figure 1 and 2).

Ethics: The patient gave written consent for this case report to be presented, and the Ethics Committee of the University Clinical Center of Serbia gave permission for the presentation (number of approval 192-22).

DISCUSSION

Similar cases of bilateral ring-shaped opacities in the cornea have been published earlier, but none of them corresponds to the case that we

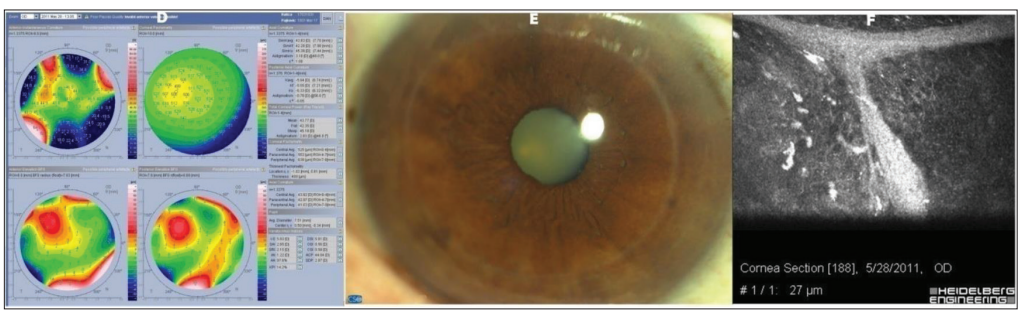


Figure 1. Topography, slit lamp photography and Heidelberg retinal tomography of the right eye

Received • Примљено: July 6, 2025
Accepted • Прихваћено: November 12, 2025
Online first: November 28, 2025

Correspondence to:
Marija BOŽIĆ
Pasterova 2
11000 Belgrade
Serbia
ammilovic@gmail.com

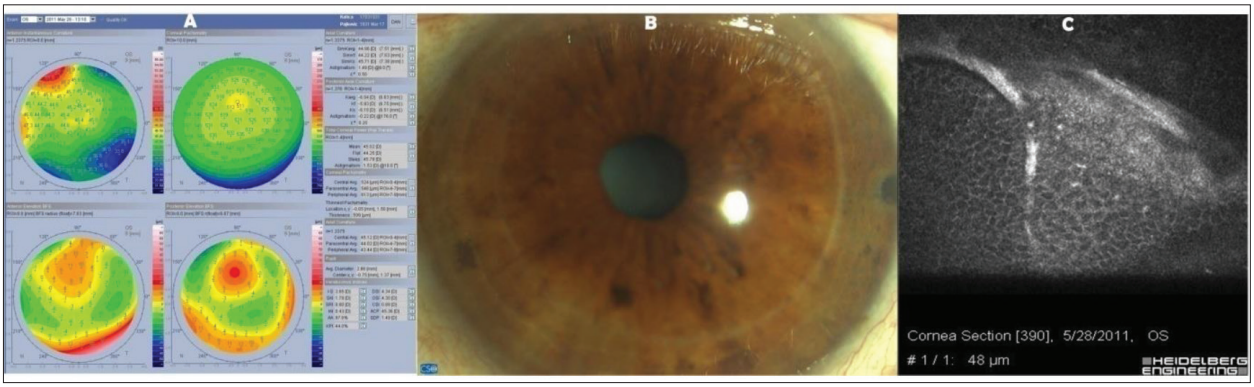


Figure 2. Topography, slit lamp photography and Heidelberg retinal tomography of the left eye

are presenting [1, 2]. Opacities in the form of geometrically regular rings on the periphery of the cornea in the described case look almost like scars from corneal transplant, but the patient did not undergo this surgery. Some of the similar peripheral corneal opacities are part of the clinical picture of the well-known corneal dystrophies [3, 4, 5]. Common feature about these previously described corneal rings is that they become visible at older age and are non-progressive. In the case that we present, we do not have data on when the clouding of the cornea appeared, because the patient did not even register them considering that they do not affect visual acuity or cause any symptoms.

We do not have an answer to the question of why our patient developed bilateral, symmetrical, almost geometrically regular hazy corneal rings. Considering that the patient has no other ophthalmological problems apart from cataracts, it is unlikely that a cornea replacement will be planned, so we cannot expect a histological answer to our question. In this case, we would like to initiate a discussion of our fellow ophthalmologists who would share their experiences and opinion.

Conflict of interests: None declared.

REFERENCES

1. Uddaraju M, Mascarenhas J, Das M, Prajna NV. A case of bilateral, multiple, symmetric, concentric ring-shaped opacities in the cornea. JAMA Ophthalmol. 2015;133(4):483–4. [DOI: 10.1001/jamaophthalmol.2014.5379] [PMID: 25569602]
2. Petrovic T, Stanojlovic S. Tomographic changes after corneal collagen cross-linking for progressive keratoconus – one-year follow-up study. Srp Arh Celok Lek. 2022;150(7–8):445–50. [DOI: 10.2298/SARH200731062P]
3. Melles GR, de Séra JP, Eggink CA, Cruysberg JR, Binder PS. Bilateral, anterior stromal ring opacity of the cornea. Br J Ophthalmol. 1998;82(5):522–5. [DOI: 10.1136/bjo.82.5.522] [PMID: 9713059]
4. Megalla M, Li E, Branden P, Chow J. Bilateral idiopathic corneal opacity: A report of Ascher ring and a review of the literature. Am J Ophthalmol Case Rep. 2021;22(23):101176. [DOI: 10.1016/j.ajoc.2021.101176] [PMID: 34368499]
5. Nofal N, Darvish-Zargar M, Teboul B. A case of symmetric bilateral ring-shaped corneal opacities. Can J Ophthalmol. 2022;57(2):e42–e44. [DOI: 10.1016/j.jcjo.2021.07.006] [PMID: 34419420]

Мистериозни прстенови на рожњачи

Марија Божић, Тања Калезић, Весна Марић, Бојана Дачић Крњаја

Универзитет у Београду, Медицински факултет, Универзитетски клинички центар Србије, Клиника за очне болести, Београд, Србија

САЖЕТАК

Увод Приказујемо случајни налаз необичних прстенастих билатералних замућења на иначе провидним рожњачама код особе без других офталмолошких болести осим катаракте. Клинички налази, *in vivo* конфокална микроскопија и налази топографије рожњаче не одговарају ниједној описаној дегенерацији рожњаче, па је циљ ове презентације консултација са колегама офталмолозима у вези са пореклом и значајем описаних промена.

Приказ случаја „Мистериозни прстен рожњаче" је случајно уочен као билатерални, асимптоматски, готово савршено округлог облика, беле боје и смештен уз лимбус. Одговарајућа дијагностика није дала одговор на порекло ових симетричних замућења.

Закључак Представљамо раније неописана симетрична билатерална замућења рожњаче, у потрази за одговором на питање да ли је неко од наших колега офталмолога имао сличне случајеве у својој пракси или поседује сазнања о каквој врсти поремећаја се ради.

Кључне речи: рожњача; замућење; дистрофија

DOI: https://doi.org/10.2298/SARH250209080J
UDC: 617.731-002

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Evaluation of macular and optic nerve head changes in young female patients with unilat-eral optic neuritis using optic coherence tomography angiography – two case reports

Miroslav Jeremić[1,2], Marija Božić[1,2], Tanja Kalezić[1,2], Dolika D. Vasović[1], Aleksandar Risimić[1]

[1]University Clinical Centre of Serbia, Clinic of Ophthalmology, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction Optic neuritis (ON) is described as an inflammation of the optic nerve which leads to the sudden loss of vision taking place over the course of several hours or days. Optical coherence tomography angiography (OCT-A) is a non-invasive imaging tool which can be used for quantitative measurements of the microvascular changes in the retinochoroidal layers and optic nerve head.

Outlines of cases We present two case reports of Serbian patients with unilateral ON whose macular and optic nerve head parameters were observed using OCT-A up to six months following initial diagnosis and the administration of corticosteroid therapy. Patient 1 was a 23-year-old female with ON in her right eye. Patient 2 was also a 23-year-old female with ON in her right eye. Retinal nerve fiber layer thickness and the radial peripapillary capillary plexus density were significantly lower in the affected *vs*. healthy eyes in both whole image scan area and optic nerve head ($p < 0.05$). Central macular thickness was significantly lower in the affected *vs*. healthy eyes ($p < 0.05$). Both superficial capillary plexus (SCP) and deep capillary plexus (DCP) vessel densities in the fovea were significantly lower in the affected *vs*. healthy eyes ($p < 0.05$). SCP vessel density was also lower in the macula of the affected eyes ($p < 0.05$). Significant differences related to the size of foveal avascular zone and selected flow area were not found ($p > 0.05$).

Conclusion ON leaves permanent structural changes which can be detected using OCT-A even in patients with fully recovered visual acuity. Therefore, OCT-A should be included more in our daily clinical practice since it can provide useful data for the prognosis of various neuro-ophthalmological diseases and conditions.

Keywords: optic neuritis; OCT-A; optic nerve head; macula; RNFL; vessel density

INTRODUCTION

Optic neuritis (ON) is described as an inflammation of the optic nerve which leads to the sudden loss of vision taking place over the course of several hours or days [1]. It is mostly idiopathic; however, it can be associated with a wide range of conditions such as demyelinating lesions, autoimmune disorders, infectious and inflammatory conditions, trauma, vascular insufficiency, metastases, toxins, or nutritional deficiencies [2]. Optical coherence tomography angiography (OCT-A) (Figures 1 and 2) is a non-invasive imaging tool which enables high-resolution imaging of the bloodstream in various layers of the retina, generating three-dimensional images, therefore allowing quantitative measurements of the microvascular changes in the retinochoroidal layers and optic nerve head [3].

Here we present two case reports of young female patients with unilateral ON whose macular and optic nerve head parameters were observed using OCT-A up to six months following initial diagnosis and the administration of corticosteroid therapy.

REPORTS OF CASES

Two female patients, both aged around 23 years, presented to the University Clinical Centre of Serbia Eye Hospital due to the sudden loss of vision followed by pain during eye movement and reduced color vision in the right eye. No previous medical history was reported. Patients were taking no medications. Upon admission, they underwent a complete ophthalmological examination and diagnosis. Best-corrected visual acuities (BCVA) were 20/640 and 20/40 in the right, affected eyes, with both having 20/20 in the left, healthy eyes. The intraocular pressures were normal, ranged 14–18 mmHg. Ocular movements were in full range but associated with pain. A relative afferent pupillary defect was present in the affected eyes. The anterior segment examination was normal, without any pathological findings. The posterior segment examination showed a normal macula, peripheral retina, and optic nerve head in both patients. Visual field testing (VFT) of the affected eyes showed significant defects in all four quadrants. The systemic and neurological examinations were within normal limits. Both patients received methylprednisolone

Received • Примљено: February 9, 2025
Revised • Ревизија: October 13, 2025
Accepted • Прихваћено: October 14, 2025
Online first: October 22, 2025

Correspondence to:
Miroslav JEREMIĆ
Clinic of Ophthalmology
University Clinical Centre of Serbia
Dunavski kej 9
11158 Belgrade
Serbia
mineolis1@yahoo.com

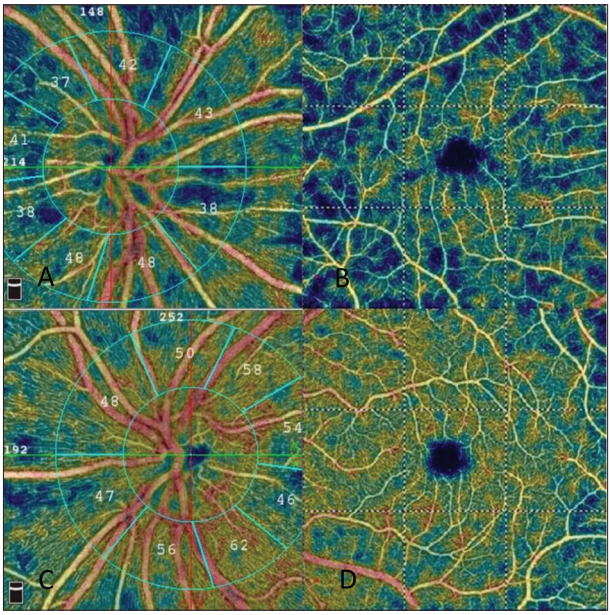


Figure 1. Optical coherence tomography angiography (OCT-A) images of the first patient; images to the left (A, C) represent radial peripapillary capillary vessel density of the right eye (A), left eye (C); images to the right (B, D) represent OCT-A macular superficial layer of the right affected eye B, and the left eye D

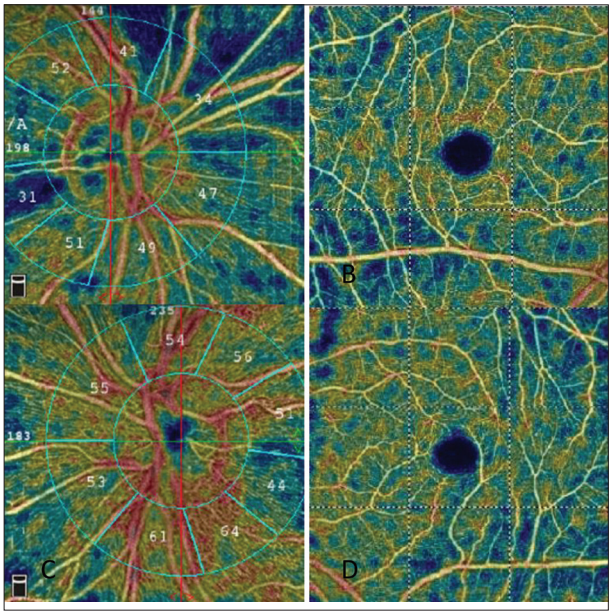


Figure 2. Optical coherence tomography angiography (OCT-A) images of the second patient; images to the left (A, C) represent radial peripapillary capillary (RPC) vessel density of right eye A, left eye C; images to the right (B, D) represent OCT-A macular superficial layer of the right affected eye B, and the left eye D

Table 1. Quantitative optical coherence tomography and optical coherence tomography angiography parameters of the affected and healthy eyes in two patients with unilateral optic neuritis

Parameters	Affected eye (case 1) (mean ± SD)	Healthy eye (case 1) (mean ± SD)	Affected eye (case 2) (mean ± SD)	Healthy eye (case 2) (mean ± SD)
FAZ (mm^2)	0.187 ± 0.004	0.199 ± 0.014	0.293 ± 0.020	0.283 ± 0.005
CMT (μm)	232.85 ± 3.976	242.28 ± 2.058	216.7 ± 1.253	229.28 ± 1.704
SCP vessel density in the macula (%)	38.987 ± 2.564	50.012 ± 3.132	46.714 ± 0.786	51.357 ± 2.702
SCP vessel density in the fovea (%)	16.163 ± 1.316	20.825 ± 2.526	12.312 ± 2.395	18.96 ± 1.957
DCP vessel density in the macula (%)	50.712 ± 8.952	55.425 ± 4.953	55.014 ± 2.554	53.501 ± 5.365
DCP vessel density in the fovea (%)	35.25 ± 4.198	41.47 ± 4.804	33.25 ± 1.168	37.47 ± 1.514
Selected flow area (mm^2)	2.244 ± 0.059	2.259 ± 0.059	2.286 ± 0.057	2.313 ± 0.042
RPC density – whole image (%)	50.085 ± 2.432	58.171 ± 1.151	52.728 ± 2.002	57.814 ± 1.007
RPC density – PNO (%)	56.83 ± 3.973	63.72 ± 1.332	63.55 ± 2.597	61.157 ± 2.505
RNFL thickness (μm)	82.25 ± 5.47	124 ± 2.97	86.14 ± 4.18	120.14 ± 2.67

FAZ – foveal avascular zone; CMT – central macular thickness; SCP – superficial capillary plexus; DCP – deep capillary plexus; RPC – radial peripapillary capillary; PNO – *papilla nervi optici*; RNFL – retinal nerve fiber layer

(1000 mg) in the form of pulse therapy intrathecally with gradual dose reduction. Significant recovery of BCVA in the affected eyes and reduction of defects in VFT were observed a few days following corticosteroid administration. Patients were discharged from the Clinic with oral prednisone therapy and scheduled for the first check-up one month after initial treatment.

A month later, at the first post-discharge check-up, OCT-A images of the macula and optic nerve head were captured for the first patient (Figure 1) as well as for the second patient (Figure 2) in both affected and healthy eyes using the AngioVue OCT-A system version 2017.1 (Optovue Inc., Fremont, CA, USA). Parameters analyzed using OCT-A (Figures 2 and 4) were as follows: 1. central macular thickness (CMT), 2. foveal avascular zone (FAZ) size, 3. macular flow using the automatic "flow" analysis function in a selected area of 3.144 mm^2 centered on the fovea, 4. superficial (SCP) and deep capillary plexus (DCP) density in the macula (scanning area 6 × 6 mm centered on the fovea) and fovea, 5. retinal nerve fiber layer (RNFL) thickness (Figures 2 and 4), and 6. radial peripapillary capillary (RPC) plexus density in both affected and healthy eyes (whole image and inside disc vessel density). Whole-image vessel density is calculated from the entire 4.5 × 4.5 mm scan field centered on the optic disc. Inside disc vessel density refers to the area inside an ellipse fitted to the optic disc boundary [4]. Both patients were followed during a period of six months. Regular check-ups were performed on a monthly basis with BCVA, VFT, and OCT-A of the macula and optic nerve head in both affected and healthy eyes being checked each time. The differences were tested using a paired-samples t-test. Statistical analysis was performed using IBM SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY, USA), and the difference was considered significant if $p < 0.05$. All results are presented in Table 1 (mean ± standard deviation).

We did not observe a significant difference related to the size of the FAZ (Patient 1: $t(7) = 3.047$, $p > 0.05$; Patient 2: $t(6) = 1.332$, $p > 0.05$) and selected flow area (Patient 1: $t(7) = 1.074$, $p > 0.05$; Patient 2: $t(6) = 0.961$, $p > 0.05$) in the affected *vs*. healthy eyes. However, CMT was significantly thinner in the affected *vs*. healthy eyes in both patients (Patient 1: $t(7) = 8.496$, $p < 0.05$; Patient 2: $t(6) = 26.14$, $p < 0.05$). Also, SCP vessel density was significantly lower in both the macula (scanning area 6 × 6 mm) (Patient 1: $t(7) = 6.705$, $p < 0.05$; Patient 2: $t(6) = 4.143$, $p < 0.05$), and the fovea (Patient 1: $t(7) = 4.224$, $p < 0.05$; Patient 2: $t(6) = 11.136$, $p < 0.05$). DCP vessel density was significantly lower in the fovea of the affected eyes (Patient 1: $t(7) = 3.623$, $p < 0.05$; Patient 2: $t(6) = 6.073$, $p < 0.05$). RPC plexus density (whole image) was significantly lower in the affected *vs*. healthy eyes (Patient 1: $t(7) = 11.925$, $p < 0.05$; Patient 2: $t(6) = 5.768$, $p < 0.05$). RPC plexus density of the optic nerve head was significantly lower in the affected *vs*. healthy eye in one patient (Patient 1: $t(7) = 4.946$, $p < 0.05$; Patient 2: $t(6) = 1.522$, $p > 0.05$). RNFL thickness was significantly lower in both patients in the affected *vs*. healthy eyes (Patient 1: $t(7) = 20.714$, $p < 0.05$; Patient 2: $t(6) = 18.89$, $p < 0.05$). During this period of time, BCVA of the affected eyes showed complete recovery of 20/20 in both patients, and VFT results were far better compared to those done previously.

Ethics: Written informed consent was obtained from the patients for publication of this case report and any accompanying images.

DISCUSSION

In this study we investigated vascular parameters of the macula and optic nerve head using OCT-A in two young female patients with unilateral ON up to six months following initial diagnosis and the administration of corticosteroid therapy. Although BCVA in the affected eyes improved significantly, reaching 20/20 at the end of the follow-up period in both patients, permanent structural changes affecting different retinal layers and the optic nerve head were observed using OCT-A.

Previous studies have shown that although patients with a history of ON associated with multiple sclerosis (MS) did not have any functional visual loss, structural neurodegeneration could be demonstrated in the affected eye. RNFL thinning in the inferior or temporal sector was independently associated with ON [5]. Recent studies have also indicated the importance of RPC plexus density reduction, as an early event in MS, which may be relevant as a potential biomarker of disease pathology [6]. The reduction of SCP vessel density in both the macula and peripapillary region in relapsing-remitting MS individuals with or without ON, being more pronounced in ON patients, has also been demonstrated [7]. Another study showed that the vessel density of SCP is significantly lower in ON eyes of both MS and patients with neuromyelitis optica spectrum disorder (NMOSD) than in non-ON eyes, while the density of DCP is significantly higher in MS+ON and MS-ON patients compared to the healthy controls. The difference was not observed when comparing NMOSD+ON and NMOSD-ON patients with healthy controls [8]. ON may cause not only retinal structural damage, but also a decreased retinal perfusion, even in patients with good visual acuity following treatment for ON [9]. Other studies support these patterns of reduced retinal SCP vessel density which correlate with reduced visual function, longer disease duration, and higher levels of global disability in MS patients with ON history [10, 11].

Based on the results of our study, RNFL thickness and the RPC plexus density were significantly lower in the affected *vs.* healthy eyes in both the whole-image scan area and optic nerve head. CMT was significantly lower in the affected *vs.* healthy eyes in both patients. Also, SCP and DCP vessel densities in the fovea were significantly lower in the affected *vs.* healthy eyes. Both patients showed significantly lower SCP vessel density in the macula of the affected eyes.

It can be concluded that even though visual acuity fully recovered following corticosteroid therapy, ON leaves permanent structural changes which can be detected using OCT-A. Therefore, OCT-A should be included more in our daily clinical practice since this powerful imaging tool can provide useful data for the prognosis of various neuro-ophthalmological diseases and conditions. To the best of our knowledge, this is the first study designed to investigate vascular parameters of the macula and optic nerve head using OCT-A in patients with unilateral ON.

ACKNOWLEDGMENT

Authors' contributions: MJ, MB, and TK analyzed and interpreted the patient data. MJ, MB, and DV were involved in conceptualization and writing of the original manuscript. DV and AR were responsible for manuscript review and preparation of the study for publication. All authors read and approved the final manuscript.

Conflict of interest: None declared.

REFERENCES

1. Hawkes CH, Giovannoni G, Lechner-Scott J, Levy M, Yeh A. Treatment of first and subsequent attacks in multiple sclerosis. Mult Scler Relat Disord. 2025;101:106665. [DOI: 10.1016/j.msard.2025.106665] [PMID: 40803050]
2. Jugnet M, David T, Pierre M, Valentino R, Mehdaoui H, Merle H. Therapeutic plasma exchange for severe optic neuritis in a region with a high prevalence of neuromyelitis optica. Ophthalmol Ther. 2025;14(10):2469–79. [DOI: 10.1007/s40123-025-01216-w] [PMID: 40782297]
3. Xie J, Ye L, Yang J, Zhong Y, Xu P, Gao B, et al. Comparison of coronary angiographic-derived and intracoronary imaging-based fractional flow reserve in assessing coronary artery stenosis severity: a systematic review and network meta-analysis. J Evid Based Med. 2025;18(3):e70054. [DOI: 10.1111/jebm.70054] [PMID: 40810699]
4. Sihota R, Shakrawal J, Azad SV, Kamble N, Dada T. Circumpapillary optical coherence tomography angiography differences in perimetrically affected and unaffected hemispheres in primary open-angle glaucoma and the preperimetric fellow eye. Indian J Ophthalmol. 2021;69(5):1120–6. [DOI: 10.4103/IJO.IJO_1191_20] [PMID: 33913845]
5. Uctepe F, Orenc P, Gungel H, Toprak UE. Evaluation of optical coherence tomography findings in patients with multiple sclerosis and glaucoma. Int Ophthalmol. 2024;44(1):226. [DOI: 10.1007/s10792-024-03099-5] [PMID: 38758396]
6. Liu J, Song S, Gu X, Li H, Yu X. Microvascular impairments detected by optical coherence tomography angiography in multiple sclerosis patients: a systematic review and meta-analysis. Front Neurosci. 2023;16:1121899. [DOI: 10.3389/fnins.2022.1121899] [PMID: 36711144]
7. El Ayoubi NK, Ismail A, Fahd F, Younes L, Chakra NA, Khoury SJ. Retinal optical coherence tomography measures in multiple sclerosis: a systematic review and meta-analysis. Ann Clin Transl Neurol. 2024;11(9):2236–53. [DOI: 10.1002/acn3.52165] [PMID: 39073308]
8. Rogaczewska M, Michalak S, Stopa M. Macular vessel density differs in multiple sclerosis and neuromyelitis optica spectrum disorder: an optical coherence tomography angiography study. PLoS One. 2021;16(6):e0253417. [DOI: 10.1371/journal.pone.0253417] [PMID: 34138942]
9. Cennamo G, Solari D, Montorio D, Scala MR, D'Andrea L, Tranfa F, et al. The role of OCT-angiography in predicting anatomical and functional recovery after endoscopic endonasal pituitary surgery: a 1-year longitudinal study. PLoS One. 2021;16(12):e0260029. [DOI: 10.1371/journal.pone.0260029] [PMID: 34855775]
10. Murphy OC, Kwakyi O, Iftikhar M, Zafar S, Lambe J, Pellegrini N, et al. Alterations in the retinal vasculature occur in multiple sclerosis and exhibit novel correlations with disability and visual function measures. Mult Scler. 2020;26(7):815–28. [DOI: 10.1177/1352458519845116] [PMID: 31094280]
11. Marjanović I, Marić V, Božić M. Optical coherent tomography with angiography in glaucoma. Srp Arh Celok Lek. 2023;151(11–12):725–9. [DOI: 10.2298/SARH230211096M]

Процена промена у макули и глави оптичког нерва код младих болесница са унилатералним оптичким неуритисом коришћењем оптичке кохерентне томографске ангиографије – приказ две болеснице

Мирослав Јеремић[1], Марија Божић[1,2], Тања Калезић[1,2], Долика Д. Васовић[1], Александар Рисимић[1]

[1]Универзитетски клинички центар Србије, Клиника за очне болести, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод Оптички неуритис представља запаљење оптичког нерва које доводи до наглог губитка вида, а развија се током неколико сати или дана. Оптичка кохерентна томографска ангиографија (*OCT-A*) неинвазивна је метода снимања која се може користити за квантитативно мерење микроваскуларних промена у ретинохориоидалним слојевима и глави оптичког нерва.

Приказ болесникâ Приказујемо два случаја из Србије. Код обе болеснице, старости 23 године, дијагностикован је оптички неуритис на десном оку и праћени су параметри макуле и главе оптичког нерва методом *OCT-A* током периода од шест месеци након постављања почетне дијагнозе и примене кортикостероидне терапије. Дебљина слоја нервних влакана ретине и густина радијалног перипапиларног капиларног сплета биле су значајно ниже на захваћеном оку у поређењу са здравим, како на целокупно посматраној површини снимка, тако и у пределу главе оптичког нерва ($p < 0,05$). Централна дебљина макуле била је значајно мања на захваћеном оку ($p < 0,05$). Густина крвних судова површинског капиларног сплета и дубоког капиларног сплета у фовеи била је значајно нижа на захваћеном у односу на здраво око ($p < 0,05$). Густина крвних судова површинског капиларног сплета била је такође нижа у макули захваћеног ока ($p < 0,05$). Нису утврђене значајне разлике у величини фовеалне аваскуларне зоне нити у изабраном подручју протока ($p > 0,05$).

Закључак Оптички неуритис оставља трајне структурне промене које се могу открити методом *OCT-A* чак и код болесника са потпуно опорављеном видном оштрином. Стога, *OCT-A* треба чешће укључивати у свакодневну клиничку праксу, јер може пружити драгоцене податке за прогнозу различитих неуроофталмолошких болести и стања.

Кључне речи: оптички неуритис; *OCT-A*; глава оптичког нерва; жута мрља; дебљина слоја нервних влакана ретине; густина крвних судова

DOI: https://doi.org/10.2298/SARH250906089P
UDC: 612.821.3

REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Primitive reflexes in developing and adult brain – from intellectual disability to dementia

Aleksandra Pavlović[1], Aleksandra Đurić-Zdravković[1], Maja Milovanović[1,2], Jelena Đorđević[3,4], Ružica Zdravković-Parezanović[1], Dragan Pavlović[1]

[1]University of Belgrade, Faculty of Special Education and Rehabilitation, Belgrade, Serbia;
[2]Institute of Mental Health, Belgrade, Serbia;
[3]University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia;
[4]Clinic of Neurology and Psychiatry for Children and Youth, Belgrade, Serbia

SUMMARY
Primitive reflexes are involuntary motor responses elicited by sensory stimuli, playing essential roles in feeding, survival, and protection during the neonatal period. Although primitive reflexes are grounded in the central nervous system integrity and are crucial for motor development and sensory processing, they are progressively inhibited and integrated as the frontal cortex matures. The persistence of primitive reflexes beyond 12 months of age may signify underlying neurodevelopmental delays, including intellectual disability, cerebral palsy, autism spectrum disorder, and other related conditions. At the other end of the lifespan spectrum, the reemergence of primitive reflexes or frontal release signs is frequently observed in older patients with various types of dementia, reflecting diffuse cerebral dysfunction and frontal lobe lesions that impair cortical inhibition of brainstem activity. This paper aims to summarize the key clinical implications of persistent primitive reflexes in cognitively affected individuals (such as intellectual disability and dementia) at both ends of the lifespan and to compare their neurobiological bases and prognostic significance to enhance understanding of these phenomena.
Keywords: primitive reflexes; intellectual disability; dementia; motor development; cognitive development

INTRODUCTION

Primitive reflexes (PRs) are automatic motor responses triggered by sensory stimuli, essential for newborns' feeding, survival, and protection [1, 2, 3]. Emerging around 25–26 weeks of gestation, these reflexes become fully functional at birth and dominate infant motor patterns during the first months of life [4]. Although PRs are grounded in central nervous system (CNS) integrity and crucial for motor and sensory development, their persistence beyond 12 months of age may indicate neurodevelopmental delays such as intellectual disability (ID), cerebral palsy (CP), autism spectrum disorder (ASD), and related conditions [3–7].

At the other end of the lifespan spectrum, the intriguing reemergence of primitive reflexes, also known as frontal release signs, is frequently observed in patients with various dementias. This finding is believed to reflect diffuse cerebral dysfunction and frontal lobe lesions that diminish cortical inhibition of brainstem activity [8]. However, PRs can also be observed in healthy elderly individuals, suggesting they may represent normal physiological aging and raising questions about their clinical significance. Nevertheless, a recent meta-analysis demonstrated that PRs in the elderly are strongly associated with an increased risk of dementia, underscoring the importance of their careful assessment during routine physical examinations of patients with cognitive decline [8].

This paper aims to summarize the key clinical implications of persistent PRs in cognitively challenged individuals with ID and dementia at opposite ends of the lifespan and to compare their neurobiological bases and prognostic significance to enhance understanding of these phenomena.

PRIMITIVE REFLEXES IN TYPICAL DEVELOPMENT

Primitive reflexes are involuntary motor responses that enable infants to interact with their environment and serve as foundational mechanisms for developing voluntary movement, supporting maturation of the motor and sensory cortex as well as the temporal–parietal–occipital association area [2, 9, 10]. During typical development (TD), brainstem-mediated PRs are gradually suppressed, integrated, and replaced by cortically controlled voluntary movements, reflecting normal CNS maturation [11–14]. As motor development advances, goal-directed actions and communicative gestures emerge through the inhibition of specific PRs, particularly those involving the hands and mouth [11, 15]. This process depends on the frontal neocortex, which exerts top-down control to inhibit PR pathways [10–13].

Received • Примљено: September 6, 2025
Revised • Ревизија: November 21, 2025
Accepted • Прихваћено: November 24, 2025
Online first: November 28, 2025

Correspondence to:
Aleksandra PAVLOVIĆ
Visokog Stevana 2
11000 Belgrade, Serbia
aleksandra3003@yahoo.com

The presence of PRs can be readily assessed in clinical practice as part of standard neurological examinations in both pediatric and adult populations; moreover, specific assessment scales have been developed for children [11, 12, 14]. Most of the over 20 PRs, with major ones listed in Table 1, typically disappear by 4–6 months postnatally, allowing the development of voluntary movements [1, 2, 3].

Retained PRs are relatively common in TD children, with prevalence rates among preschoolers ranging from 43.2% to 80%, varying by assessment methods [9, 15–19]. According to some data, over 90% of healthy children aged 4–6 years exhibit at least one PR to some degree [15]. These findings highlight significant variability in CNS maturation, extending over broader timeframes than previously recognized. The underlying determinants, likely involving genetic and environmental factors, remain largely unknown [1, 2, 3].

PRIMITIVE REFLEXES IN NEURODEVELOPMENTAL DISORDERS

The phenomenon of retained PRs is attributed to brain functional dysconnectivity caused by maturational delays in specific cortical networks. This can lead to compensatory growth in other networks, resulting in asynchronous development and inconsistent functional abilities [10]. When PRs fail to be suppressed, they continue to drive bottom-up interference with the cortex, hindering brain maturation and the establishment of top-down regulatory control. This may cause widespread dysregulation of the nervous, immune, and endocrine systems [20]. Persistence of PRs beyond the first year of life typically indicates psychomotor developmental delays or neurological impairments. This has been well documented in conditions such as CP, ASD, ID, attention-deficit/hyperactivity disorder (ADHD), developmental coordination disorder, Tourette's syndrome, and learning disorders [1, 3, 6, 11, 20–26]. Retained PRs negatively impact motor skill development, cognitive processing, and emotional regulation [5, 27]. When these reflexes persist beyond their typical integration period, they may interfere with a child's ability to coordinate movements, focus attention, and regulate emotional responses effectively [5, 16, 27].

Frontal lobe maturation delays, the primary cause of persistent PRs, are also linked to delayed postural reflex development, hindering sensory-motor milestones such as crawling and walking. Additionally, these delays often coincide with impaired executive functions, a common feature of neurodevelopmental disorders [10]. Early detection of persistent PRs may aid in diagnosing neurodevelopmental disorders, but its reliability is debated [9]. The persistence of PRs in individuals with ID and other neurodevelopmental disorders can facilitate early identification of at-risk children, clarify underlying pathological mechanisms, and improve understanding of cognitive impairment pathways. Additionally, it supports targeted selection for early intervention programs [3, 28].

Primitive reflexes in intellectual disability

Limited epidemiological data exist on PRs in individuals with ID because this group is often excluded from studies or not distinctly separated from other neurodevelopmental disorders [15, 20–26, 29]. A recent review noted frequent retention of the Moro reflex in individuals with ID, including those with Down syndrome, athetoid CP, and occasionally spastic CP. However, as the primary sources are not in English, detailed data remain limited [5, 30, 31].

Besides delayed cortical maturation and network dysfunction, especially in the frontal lobes, persistent PRs have also been associated with abnormal asymmetric development of the brain hemispheres [23, 32]. The causes of retained PRs often remain unclear, but may include insufficient external stimuli essential for TD and exposure to harmful environmental factors [9, 12]. While structural and functional neuroimaging studies have investigated the mechanisms of PRs in elderly populations with neurological disease, no comparable research has been conducted in individuals with ID.

Clinical significance of persistent primitive reflexes in intellectual disability

Retained PRs often signify maturational delay, manifested by structural and functional changes, alongside motor and cognitive delays [27]. High-risk newborns frequently exhibit abnormal, asymmetrical, or absent reflex response [10]. Children with ID typically show reduced motor skills relative to age-matched peers and neurodevelopmental norms, correlating with ID severity and manual dexterity [33, 34]. In isolated ID cases, PR persistence is found in 24.6% of cases and is more common in comorbidities like ASD or CP. The prevalence of retained PRs increases with ID severity, being higher in severe and profound than in mild or moderate cases [29].

An early study reported a higher prevalence of the palmomental (47% *vs*. 7%) and snout reflexes (14% *vs*. 0%) in individuals with Down syndrome compared to healthy controls [35]. However, with a mean age of 37 years in the Down syndrome group, these findings rather represent reemergence of PRs than their retention [8].

In ASD, Minderaa et al. [29] observed increased prevalence of snout and visual rooting reflexes (VRR) compared to TD controls. Teitelbaum et al. (2002) [36] noted persistence of some PRs in ASD cases, particularly the asymmetrical tonic neck reflex. De Buildt et al. [37] found the VRR more common in ASD individuals with ID (43.9%) than in those with ID alone (24.6%), and its persistence correlated with lower global cognition and adaptive functioning. Chinello et al. [11] reported greater PRs persistence in 12–17-month-old infants whose parents exhibited higher subclinical autistic traits.

Healy et al. [13] found a higher prevalence of the snout and VRR in children with ASD compared to peers with developmental delays or TD, matched for age (4–6 years). They proposed that persistence of these reflexes may serve as developmental biomarkers for ASD, potentially

Table 1. Major primitive reflexes in infancy, their time-line and purpose [1, 2, 3]

PR	Time-line	Purpose
Moro	From 34th GW to 3–4 months post birth	Instant arousal of survival systems
Palmar grasp	From birth until 5–6 months post birth	Aid clinging (protection from fall)
Walking/stepping	From birth until 6 weeks	Preparation for voluntary locomotion
Doll's eye (oculocephalic reflex)	Disappears within a week or two after birth	Gaze fixation on stationary objects
Rooting (tactile or visually evoked)	From 28 GW to 3–4 months of age	Food finding; breastfeeding
Sucking	Appears at 28 GW and disappears within 24 months	Feeding
Snout	Appears at 26 GW and disappears within 3–6 months	Feeding
Glabellar	Disappears within 3–6 months	Blinking in response to tactile stimulation – eyes protection
Asymmetrical tonic neck reflex	From 18 GW to 3–6 months after birth	Assists through birth canal and in developing cross pattern movements
Galant	From birth until 3–9 months	Assists baby with birth process, crawling and creeping

PR – primitive reflex; GW – gestational week

Table 2. Primitive reflexes across the lifespan – prevalence, clinical significance, associated conditions [1, 2, 3, 8, 40]

Lifespan segment	Prevalence	Clinical significance	Associated conditions
Prenatal	Present in all healthy fetuses since the third trimester	Normal finding	/
Infancy and toddlerhood (birth to 2 years)	Present in all healthy infants until 6 months	Normal finding within 6 months after birth	May indicate maturation delay if present beyond 6/24 months
Early childhood (2–6 years)	Present in 40–90% healthy children	Isolated PRs can be detected in healthy subjects	May indicate maturation delay or neurodevelopmental disorders, cerebral palsy
Middle childhood and adolescence (6–18 years)	Up to 65% of healthy individuals	Isolated PRs can be detected in healthy subjects	May indicate brain injury affecting frontal lobe or diffuse cortical lesions
Adulthood (18–65 years)	6–47% of neurological intact individuals	Isolated PRs can be detected in healthy subjects	May be sign of brain injury or development of progressive neurological condition
Late adulthood (older than 65 years)	40–100% of neurologically and cognitively intact individuals	Isolated PRs can be detected in healthy subjects but correlates with cognitive status; may be warning sign of cognitive deterioration	May be sign of brain injury or development of progressive neurological condition

PRs – primitive reflexes

facilitating earlier diagnosis, especially in cases with comorbid ID [13].

In a cohort of 81 infants with ID without motor disturbances, Futagi et al. [38] observed tendencies toward retained Galant reflex, asymmetric tonic neck reflex, and plantar grasp. An earlier prospective study of 53 infants with developmental delays but no physical disabilities reported delayed postural adjustment reflexes relative to chronological age [39].

PRIMITIVE REFLEXES ACROSS THE LIFESPAN

In Table 2, the reported prevalence, clinical significance, and associated conditions of PRs across the lifespan are summarized [1, 2, 3, 40]. While PRs are typically present during early childhood as part of normal neurodevelopment, reflecting immature cortical control, particularly within the frontal lobes, their gradual suppression within the first year after birth signifies cortical maturation and enables the development of voluntary movements. Persistence of these reflexes beyond infancy or their reemergence in advanced aging often indicates delayed or disrupted frontal cortical development or neurodegeneration predominantly affecting cortical networks and their connections, sometimes heralding the onset of cognitive decline [41].

PRIMITIVE REFLEXES IN NORMAL AGING

Persistent PRs, including the palmomental reflex, snout reflex, and the sucking reflex can be detected across all ages in individuals with TD, including older adults without neurological or cognitive impairments, with their reappearance increasing with age [8, 42]. Early studies reported that about one in six healthy adults exhibits at least one PR, with palmomental reflex present in 6–27% of young adults (20–50 years) and 28–60% of those over 60 years [8, 10, 42]. Results of the Maastricht aging study reported at least one PR in 47% of men and 51% of those aged 25–45 years, with no associated cognitive dysfunction [41]. Snout reflex appears in 13% of individuals aged 40–57 years and 22–33% of those above 60; the sucking reflex is observed in 6% of normal individuals aged 73–93 years [8, 10, 40, 42]. Retained PRs have been detected in 33% of neurologically and cognitively healthy aging subjects (age range 45–91 years), and their presence correlates with decreased performance on cognitive tests compared to those without PRs, although suntil within the normal range [40]. This indicates that retained PRs may be a warning sign of incipient cognitive decline.

Among isolated, subtle, neurological abnormalities (ISNAs) in neurologically and cognitively healthy individuals, PRs are the most common. They have been linked to

magnetic resonance imaging (MRI) markers of small vessel disease, the most frequent form of vascular brain changes associated with cognitive decline and dementia, including deep and subcortical white matter hyperintensities, lacunar infarcts, and subcortical atrophy [43, 44, 45]. In addition, ISNAs, including PRs, also correlate with vascular risk factors such as hypertension, hypertriglyceridemia, and apolipoprotein E (APOE) ε4 carrier status [44, 46]. These findings suggest vascular brain damage as a potential cause and emphasize the clinical relevance of PRs as early indicators of underlying pathology [44, 46]. Vascular and degenerative changes accumulate over decades with no overt cognitive or functional symptoms but likely impair brain networks [44]. These changes are likely causing PRs reemergence as an epiphenomenon of covert brain damage that may precede cognitive decline [44].

PRIMITIVE REFLEXES IN DEMENTIA

The reemergence of PRs is frequently observed in conditions affecting the frontal lobes or their associative areas, including Alzheimer's disease (AD), vascular dementia, frontotemporal dementia, Parkinson's disease, and other neurodegenerative disorders [8, 45, 47]. Early studies reported PR prevalence rates of 16% in healthy controls, 32% in mild cognitive impairment, and 58% in dementia, indicating a progressive increase linked to cognitive decline [6]. Additionally, the presence of any PR increased the likelihood of other neurological signs, such as bradykinesia [46].

A recent meta-analysis of observational and cohort studies demonstrated that individuals with dementia have a four- to 16-fold higher risk of exhibiting PRs, particularly the grasp reflex, compared to healthy controls [8]. The snout reflex was the most commonly detected, present in about one half of cases, while the grasp reflex posed the highest dementia risk, suggesting links between specific PRs and frontal cortex volume or connectivity [8]. Further insights into PR reemergence come from 18F-fluorodeoxyglucose positron emission tomography studies, revealing hypometabolism in the superior frontal gyrus and putamen and implicating corticostriatal motor circuit dysfunction (supplementary motor area–putamen–thalamus) in dementia patients [48].

Clinical significance of primitive reflexes in dementia

A strong link between PRs and cognitive dysfunction is well documented. The presence of PRs correlates with poorer performance in global cognition, executive function, attention, and language in older adults, suggesting they may serve as early indicators of cognitive impairment [8]. Patients with degenerative dementia and PRs exhibit more severe cognitive and functional decline than those without PRs [48]. Notably, cerebral metabolism was lower in patients with PRs in the bilateral superior frontal gyri, bilateral putamina, and thalami, with no brain regions showing increased metabolism compared to those without, indicating distinct mechanisms of PR reemergence *vs.* persistence [48]. Analysis of individual reflexes showed similar hypometabolic brain regions, yet these did not overlap with primary affected areas in AD or frontotemporal dementia, highlighting a unique pathophysiology associated with PRs [49]. Additionally, PR incidence increases with advancing age.

The presence of ISNAs, including PRs, is observed across all mild cognitive impairment subtypes, particularly in cases involving multiple cognitive domains and carriers of the *APOE* ε4 allele. These abnormalities correlate with MRI evidence of silent small vessel disease and subcortical atrophy [49]. Cognitive decline and PR presence likely share neurodegenerative mechanisms, with prefrontal neural networks being especially susceptible to aging-related deterioration manifested as structural degradation, connectivity loss, and functional inefficiency [50].

CONCLUSION

In infants, PRs serve as evolutionary adaptations that enhance survival during early life by providing immediate protection, aiding feeding, and stimulating neural pathways crucial for subsequent motor and sensory development. As infants mature, most PRs gradually integrate into more complex behaviors that support motor control, cognitive processing, and emotional regulation. The persistence of PRs in ID remains understudied, with unclear underlying mechanisms and clinical impacts. While retained PRs may indicate neurodevelopmental disorders such as ID, many children exhibiting these reflexes develop without impairments. In adults, particularly the elderly, persistent PRs are considered pathological, signaling frontal lobe dysfunction or disrupted connectivity between frontal and other brain regions, and serve as a warning sign for potential cognitive decline.

Across the lifespan, there is strong evidence linking motor and cognitive functions both in children with developmental and intellectual delays and in adults experiencing progressive cognitive decline and dementia. CNS maturation involves a transition from brainstem-mediated reflexes to cortically controlled responses; however, vulnerability of the frontal cortex to aging, vascular, and degenerative processes leads to loss of top-down regulation.

ACKNOWLEDGMENT

This paper was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract number 451-03-137/2025-03/200096).

Ethics: The authors declare that the article was written according to ethical standards of the Serbian Archives of Medicine as well as the ethical standards of the medical facilities of each author involved.

Conflict of interest: None declared.

REFERENCES

1. Bilbilaj S, Gjipali A, Shkurti F. Measuring primitive reflexes in children with learning disorders. Eur J Multidiscip Stud. 2017;2(5):285–98. [DOI: 10.26417/ejms.v5i1.p285-298]
2. Jaiswal M, Morankar R. Understanding primitive reflexes and their role in growth and development: a review. Int Healthc Res J. 2017;1(8):243–7. [DOI: 10.26440/IHRJ/01_08/123]
3. Đurić-Zdravković A. Rani tretman i predškolsko vaspitanje dece s intelektualnom ometenošću. Beograd: Univerzitet u Beogradu – Fakultet za specijalnu edukaciju i rehabilitaciju; 2025.
4. Pavlović A. Osnove razvojne neurologije za studente Fakulteta za specijalnu edukaciju i rehabilitaciju. Beograd: Univerzitet u Beogradu – Fakultet za specijalnu edukaciju i rehabilitaciju; 2024.
5. Rashikj Canevska O, Mihajlovska M. Persistence of primitive reflexes and associated problems in children. God Zb Fil Fak Skopje. 2019;72(22):513–22. [DOI: 10.37510/godzbo1972513rc]
6. Daeng T, Noviana M, Nasaruddin F. Relationship between primitive reflex values and gross motor abilities in children with spastic cerebral palsy. J Phys Conf Ser. 2020;1529:032041. [DOI: 10.1088/1742-6596/1529/3/032041]
7. Zdravković-Parezanović R, Đurić-Zdravković A. Stres kod roditelja dece s nekim neurorazvojnim poremećajima – narativni pregled. Beogr Defektol Šk. 2024;30(2):23–39.
8. Altunkalem Seydi K, Kaya D, Yavuz I, Ontan MS, Dost FS, Isik AT. Primitive reflexes and dementia in older adults: a meta-analysis. Psychogeriatrics. 2024;24(3):688–700. [DOI: 10.1111/psyg.13098] [PMID: 38400649]
9. Calvin N, Ramli Y. Detecting neurodevelopmental problems in children aged 1–5 years using a parent-reported screening tool with primitive reflex assessment. Paediatr Indones. 2020;60(1):31–6. [DOI: 10.14238/pi60.1.2020.31-36]
10. Leisman G, Melillo R. Evaluating primitive reflexes in early childhood as a potential biomarker for developmental disabilities. J Paediatr Child Health. 2025;61(6):846–51. [DOI: 10.1111/jpc.70053] [PMID: 40196932]
11. Chinello A, Di Gangi V, Valenza E. Persistent primary reflexes affect motor acts: potential implications for autism spectrum disorder. Res Dev Disabil. 2018;83:287–95. [DOI: 10.1016/j.ridd.2016.07.010] [PMID: 27595468]
12. Zakaria Z, Van Rostenberghe H, Ramli N, Suhaimi MS, Hazlan SNH, Abdullah JM. The key aspects of neonatal and infant neurological examination. Malays J Med Sci. 2023;30(4):193–206. [DOI: 10.21315/mjms2023.30.4.16] [PMID: 37655147]
13. Healy O, Reilly E, Davies J, Lovett V, Reed P. Differential persistence of primary reflexes in children with ASD: a systematic replication. J Autism Dev Disord. 2024;54(12):4752–6. [DOI: 10.1007/s10803-022-05880-w] [PMID: 36562932]
14. Wang M, Yu J, Li H, Zhao C, Li Y, Yang X. Development of the children's primitive reflex integration assessment scale. Front Psychol. 2025;16:1495990. [DOI: 10.3389/fpsyg.2025.1495990] [PMID: 39911996]
15. Pecuch A, Gieysztor E, Wolańska E, Telenga M, Paprocka-Borowicz M. Primitive reflex activity in relation to motor skills in healthy preschool children. Brain Sci. 2021;11(8):967. [DOI: 10.3390/brainsci11080967] [PMID: 34439585]
16. Gieysztor EZ, Choińska AM, Paprocka-Borowicz M. Persistence of primitive reflexes and associated motor problems in healthy preschool children. Arch Med Sci. 2018;14(1):167–73. [DOI: 10.5114/aoms.2016.60503] [PMID: 29379547]
17. Goddard Blythe S, Duncombe R, Preedy P, Gorely T. Neuromotor readiness for school: the primitive reflex status of young children at the start and end of their first year at school in the United Kingdom. Educ 3-13. 2022;50(5):654–67. [DOI: 10.1080/03004279.2021.1895276]
18. Hickey J, Feldhacker D. Primitive reflex retention and attention among preschool children. J Occup Ther Sch Early Interv. 2022;15(1):1–13. [DOI: 10.1080/19411243.2021.1910606]
19. Rathod HP, Patel BG, Mishra AK, Somarajan S. Prevalence of active primitive reflexes and its impact on motor skills and sensory processing in preschool children. Afr J Bio Sci. 2024;6(8):3059–78. [DOI: 10.48047/AFJBS.6.8.2024.3059-3078]
20. Melillo R, Leisman G, Machado C, Machado-Ferrer Y, Chinchilla-Acosta M, Kamgang S, et al. Retained primitive reflexes and potential for intervention in autistic spectrum disorders. Front Neurol. 2022;13:922322. [DOI: 10.3389/fneur.2022.922322] [PMID: 35873782]
21. Bob P, Konicarova J, Raboch J. Disinhibition of primitive reflexes in ADHD. Front Psychiatry. 2021;12:430685. [DOI: 10.3389/fpsyt.2021.430685] [PMID: 34819879]
22. Matuszkiewicz M, Gałkowski T. Developmental language disorder and uninhibited primitive reflexes in young children. J Speech Lang Hear Res. 2021;64(3):935–48. [DOI: 10.1044/2020_JSLHR-19-00423] [PMID: 33621124]
23. Sigafoos J, Roche L, O'Reilly M, Lancioni G. Persistence of primitive reflexes in developmental disorders. Curr Dev Disord Rep. 2021;8:98–105. [DOI: 10.1007/s40474-021-00232-2]
24. Kalemba A, Lorent M, Blythe SG, Gieysztor E. The correlation between residual primitive reflexes and clock-reading difficulties in school-aged children – a pilot study. Int J Environ Res Public Health. 2023;20(3):2322. [DOI: 10.3390/ijerph20032322] [PMID: 36767689]
25. Đurić-Zdravković A, Milovanović M, Čvorović Đ, Pavlović A. Developmental coordination disorder – clinical features and treatment options. Srp Arh Celok Lek. 2024;152(11–12):638–44. [DOI: 10.2298/SARH240730081D]
26. McWhirter K, Steel A, Adams J. The association between learning disorders, motor function, and primitive reflexes in pre-school children: a systematic review. J Child Health Care. 2024;28(2):402–28. [DOI: 10.1177/13674935221114187] [PMID: 35830652]
27. Martello JM. Persistent primitive reflex and developmental delay in the school-aged child. J Nurse Pract. 2023;19(10):104767. [DOI: 10.1016/j.nurpra.2023.104767]
28. Maksimović S, Marisavljević M, Stanojević N, Ćirović M, Punišić S, Adamović T, et al. Importance of early intervention in reducing autistic symptoms and speech-language deficits in children with autism spectrum disorder. Children (Basel). 2023;10(1):122. [DOI: 10.3390/children10010122] [PMID: 36670672]
29. Minderaa RB, Volkmar FR, Hansen CR, Harcherik DF, Akkerhuis GW, Cohen DJ. Snout and visual rooting reflexes in autism. J Autism Dev Disord. 1985;15(4):409–16. [DOI: 10.1007/BF01531785] [PMID: 4077815]
30. Iiyama M, Miyajima T, Hoshika A. Developmental change of Moro reflex studied with a three-dimensional motion analysis system. No To Hattatsu. 2002;34(4):307–12. [PMID: 12134681]
31. Čičevska-Jovanova N, Rašik-Canevska O. Rana intervencija na deca so rizik. Skopje: Univerzitet „Sv. Kiril i Metodij"; 2013.
32. Duchan E. Child developmental delays and disorders: motor delay. FP Essent. 2021;510:11–6. [PMID: 34709024]
33. Vuijk PJ, Hartman E, Scherder E, Visscher C. Motor performance of children with mild intellectual disability and borderline intellectual functioning. J Intellect Disabil Res. 2010;54(11):955–65. [DOI: 10.1111/j.1365-2788.2010.01318.x] [PMID: 20854287]
34. Taylor SL, Downs SJ, Rudd JR, McGrane B, Melville CA, McGarty AM, et al. Associations between motor competence and physical activity levels of children with intellectual disabilities and/or autism spectrum disorder: Movement matters. J Intellect Disabil. 2024;28(4):976–93. [DOI: 10.1177/17446295231203764] [PMID: 37729890]
35. Sand T, Mellgren SI, Hestnes A. Primitive reflexes in Down's syndrome. J Ment Defic Res. 1983;27(Pt 1):39–44. [DOI: 10.1111/j.1365-2788.1983.tb00161.x] [PMID: 6223145]
36. Teitelbaum P, Teitelbaum OB, Fryman J, Maurer R. Reflexes gone astray in autism in infancy. J Dev Learn Disord. 2002;6:15–22.
37. de Bildt A, Mulder EJ, Van Lang ND, de With SA, Minderaa RB, Stahl SS, et al. The visual rooting reflex in individuals with autism spectrum disorders and co-occurring intellectual disability. Autism Res. 2012;5(1):67–72. [DOI: 10.1002/aur.225] [PMID: 21954217]
38. Futagi Y, Tagawa T, Otani K. Primitive reflex profiles in infants: differences based on categories of neurological abnormality. Brain Dev. 1992;14(5):294–8. [DOI: 10.1016/S0387-7604(12)80146-X] [PMID: 1456382]
39. Molnar GE. Analysis of motor disorder in retarded infants and young children. Am J Ment Defic. 1978;83(3):213–22. [PMID: 717435]
40. Camarda C, Torelli P, Pipia C, Azzarello D, Battaglini I, Sottile G, et al. Nociceptive primitive reflexes in neurologically and cognitively healthy aging subjects. Can J Neurol Sci. 2019;46(2):199–208. [DOI: 10.1017/cjn.2018.388] [PMID: 30761966]
41. Yang R, Ji F, Jiao J. CNS development and neuron regeneration. Curr Opin Genet Dev. 2025;90:102286. [DOI: 10.1016/j.gde.2024.102286] [PMID: 39637751]

42. Stephens-Sarlós E, Toth E, Ihász F, Alföldi Z, Somogyi A, Szabo A. Changes in primitive reflexes in older adults and their relationship to mental health indices: an experimental investigation. Exp Gerontol. 2024;196:112583. [DOI: 10.1016/j.exger.2024.112583] [PMID: 39284460]
43. Pavlović A, Pavlović D, Aleksić V, Šternić N. Vascular dementia: facts and controversies. Srp Arh Celok Lek. 2013;141(3–4):247–55. [DOI: 10.2298/SARH1304247P] [PMID: 23745353]
44. Camarda C, Torelli P, Camarda R, Battaglini I, Gagliardo C, Monastero R. Isolated, subtle, neurological abnormalities in neurologically and cognitively healthy aging subjects. J Neurol. 2015;262(5):1328–39. [DOI: 10.1007/s00415-015-7716-5] [PMID: 25825125]
45. Verdelho A, Biessels GJ, Chabriat H, Charidimou A, Duering M, Godefroy O, et al. Cerebrovascular disease in patients with cognitive impairment: a white paper from the ESO dementia committee – a practical point of view with suggestions for the management of cerebrovascular diseases in memory clinics. Eur Stroke J. 2021;6(2):111–19. [DOI: 10.1177/2396987321994294] [PMID: 34414285]
46. Hogan DB, Ebly EM. Primitive reflexes and dementia. Age Ageing. 1995;24(5):375–81. [DOI: 10.1093/ageing/24.5.375] [PMID: 8669338]
47. Mijajlović MD, Pavlović A, Brainin M, Heiss WD, Quinn TJ, Ihle-Hansen HB, et al. Post-stroke dementia – a comprehensive review. BMC Med. 2017;15(1):11. [DOI: 10.1186/s12916-017-0779-7] [PMID: 28095900]
48. Matias-Guiu JA, Cabrera-Martín MN, Fernández-Matarrubia M, Moreno-Ramos T, Valles-Salgado M, Porta-Etessam J, et al. Topography of primitive reflexes in dementia: an F-18 fluorodeoxyglucose positron emission tomography study. Eur J Neurol. 2015;22(8):1201–7. [DOI: 10.1111/ene.12726] [PMID: 25926068]
49. Camarda C, Camarda R, Pipia C, Azzarello D, Grassedonio E, Sottile G, et al. Isolated, subtle neurological abnormalities in mild cognitive impairment types. Can J Neurol Sci. 2020;47(1):77–91. [DOI: 10.1017/cjn.2019.293] [PMID: 31486349]
50. Ranjbar-Slamloo Y, Chong HR, Kamigaki T. Aging disrupts the link between network centrality and functional properties of prefrontal neurons during memory-guided behavior. Commun Biol. 2025;8(1):62. [DOI: 10.1038/s42003-025-07498-x] [PMID: 39820515]

Примитивни рефлекси и мозак у развоју и одраслом добу – од интелектуалне ометености до деменције

Александра Павловић[1], Александра Ђурић-Здравковић[1], Маја Миловановић[1,2], Јелена Ђорђевић[3,4], Ружица Здравковић-Парезановић[1], Драган Павловић[1]

[1]Универзитет у Београду, Факултет за специјалну едукацију и рехабилитацију, Београд, Србија;
[2]Институт за ментално здравље, Београд, Србија;
[3]Универзитет у Крагујевцу, Факултет медицинских наука, Крагујевац, Србија;
[4]Клиника за неурологију и психијатрију за децу и омладину, Београд, Србија

САЖЕТАК

Примитивни рефлекси су невољни моторички одговори који се активирају сензорним стимулусима и имају кључну улогу у храњењу, преживљавању и заштити у неонаталном периоду. Иако су засновани на интегритету централног нервног система и неопходни у моторичком развоју и сензорном процесирању, током сазревања фронталног кортекса инхибирају се и интегришу. Њихово трајање након 12 месеци од рођења може указивати на неуроразвојно кашњење, укључујући интелектуалну ометеност, церебралну парализу, поремећај из спектра аутизма и друге поремећаје. На другом крају животног спектра поновна појава примитивних рефлекса или присуство дезинхибиционих феномена често се јавља код старијих пацијената са свим врстама деменције, што указује на дифузну церебралну дисфункцију и лезије фронталног режња које слабе кортикалну инхибицију активности мождано стабла. Циљ овог рада је да сумира главне клиничке импликације перзистентних примитивних рефлекса код особа са когнитивним изазовима (као што су интелектуална ометеност и деменција) на различитим крајевима животног спектра и да упореди њихову неуробиолошку основу и прогностичку вредност ради бољег разумевања овог феномена.

Кључне речи: примитивни рефлекси; интелектуална ометеност; деменција; моторички развој; когнитивни развој

DOI: https://doi.org/10.2298/SARH241111093B
UDC: 616.316-006.6

REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Potential pitfalls within the AJCC 8th Edition Staging System for Salivary Gland Carcinoma

Borivoj Bijelić[1], Denis Brajković[2], Momir Stevanović[3], Vladan Đorđević[3]

[1]University of Belgrade, School of Dental Medicine, Belgrade, Serbia;
[2]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia;
[3]University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia

SUMMARY
Salivary gland carcinomas (SGCs) are uncommon malignancies, representing less than 5% of all head and neck cancers. They exhibit marked variation in their histological types, as well as in clinical and biological behavior. According to the 5th edition of the World Health Organization (WHO) Classification of Head and Neck Tumours (5th ed.), in the chapter on salivary gland tumors, there are 21 recognized types of SGCs. The prognosis of patients with SGCs is currently assessed using the Tumor, Nodes, Metastasis staging system established by the American Joint Committee on Cancer (AJCC). However, recent evidence indicates that this system may lack sufficient sensitivity in predicting treatment response and survival outcomes, particularly with respect to cervical nodal involvement. Notably, the 8th edition of the AJCC staging protocol applies the same N-classification to both human papillomavirus-negative squamous cell carcinoma of the upper aerodigestive tract and SGCs, despite marked differences in biological behavior, therapeutic strategies, and clinical outcomes between these entities. The pitfalls in the 8th AJCC N-staging system for SGCs include the lack of prognostic significance of extranodal extension, the lack of consideration of parotid lymph nodes, and the significance of bilateral neck metastases, which are extremely rare in SGCs. The aim of the present narrative review was to highlight the unresolved limitations of the AJCC 8th edition.
Keywords: salivary gland carcinoma; staging; classification; pathological nodal status; depth of invasion

INTRODUCTION

Salivary gland carcinomas (SGCs) are rare malignancies, representing less than 5% of all head and neck cancers [1, 2]. According to the World Health Organization (WHO) – Global Cancer Observatory, in 2020 the global incidence was 0.59 and the mortality rate 0.23 per 100,000 per year [1, 2]. Despite their rarity, SGCs display remarkable heterogeneity in histological subtypes, with diverse clinical and biological behavior. The 5th edition of the WHO classification of SGCs recognizes 21 distinct primary malignant entities [3]. Approximately 80% of SGCs arise in the parotid gland, whereas carcinomas of the submandibular, sublingual, and minor salivary glands of the upper aerodigestive tract account for the remaining 20% [1, 2, 3]. Management typically involves surgical excision of the primary tumor, neck dissection in cases of cervical lymph node involvement, and adjuvant radiotherapy or concurrent chemoradiotherapy in advanced cases to improve overall survival and locoregional control [4].

The survival of patients with SGCs is estimated using the Tumor, Nodes, Metastasis (TNM) staging system developed by the American Joint Committee on Cancer (AJCC) [5] (Table 1). The 8th edition of the AJCC staging system for SGCs aimed to convey disease extent and stage, enabling physicians to plan treatment modalities and evaluate prognosis and outcomes [5]. Thus, the primary role of the TNM staging system is to predict survival rates and treatment outcomes. Recent evidence indicates that the current staging system for SGCs may lack sufficient sensitivity in predicting treatment and survival outcomes. The aim of the present narrative review was to highlight the unresolved pitfalls of the AJCC 8th edition.

DEPTH OF INVASION

Staging of minor SGCs of the upper aerodigestive tract currently parallels that of squamous cell carcinoma arising in the same sites, despite substantial differences in their clinical and biological behavior. In the 8th edition of the AJCC TNM classification, depth of invasion was incorporated into T-staging and introduced as a key prognostic factor for oral squamous cell carcinoma, strongly correlated with overall survival [5, 6]. Depth of invasion, defined as the vertical distance from the reconstructed mucosal surface to the deepest point of tumor invasion, is considered the primary predictor of lymph node metastasis in early-stage oral cancer [5, 6]. However, the application of depth of invasion to staging minor SGCs remains controversial. Calabrese et al. argued that, as minor SGCs are typically submucosal in origin, the concept of depth of invasion is not applicable in cases where the tumor does not infiltrate the

Received • Примљено: November 11, 2024
Revised • Ревизија: December 2, 2025
Accepted • Прихваћено: December 2, 2025
Online first: December 4, 2025

Correspondence to:
Denis BRAJKOVIĆ
University Clinical Center of Vojvodina
Clinic for Maxillofacial and Oral Surgery
Hajduk Veljkova 1–9
21000 Novi Sad, Serbia
denis.brajkovic@mf.uns.ac.rs

Table 1. The AJCC TNM Classifications for Salivary Gland Carcinomas

Primary tumor (T)	
Primary tumor (T) – major salivary glands (parotid, submandibular, and sublingual)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor ≤ 2 cm in greatest dimension without extraparenchymal extension*
T2	Tumor > 2 cm but not more than 4 cm in greatest dimension without extraparenchymal extension*
T3	Tumor > 4 cm and/or tumor having extraparenchymal extension*
T4	Moderately advanced or very advanced disease
T4a	Moderately advanced disease; Tumor invades the skin, mandible, ear canal, and/or facial nerve
T4b	Very advanced disease; Tumor invades skull base and/or pterygoid plates and/or encases carotid artery
Primary tumor (T) – minor salivary glands	
Minor salivary gland carcinomas are staged, by convention, using the mucosal tumor staging classification, according to the anatomical site of the tumor	
Regional lymph nodes – pathological N	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node ≤ 3 cm in greatest dimension and no extranodal extension (ENE [-])
N2	Metastasis in a single ipsilateral lymph node, 3 cm or smaller in greatest dimension and ENE (+); or a single ipsilateral lymph node > 3 cm but not more than 6 cm in greatest dimension and ENE (-); or metastases in multiple ipsilateral lymph nodes, none > 6 cm in greatest dimension and ENE (-); or in bilateral or contralateral lymph nodes, none > 6 cm in greatest dimension and ENE (-)
N2a	Metastasis in a single ipsilateral lymph node, 3 cm or smaller in greatest dimension and ENE (+); or a single ipsilateral lymph node > 3 cm but not more than 6 cm in greatest dimension and ENE (-)
N2b	Metastasis in multiple ipsilateral lymph nodes, none > 6 cm in greatest dimension and ENE (-)
N2c	Metastasis in bilateral or contralateral lymph node(s), none > 6 cm in greatest dimension and ENE (-)
N3	Metastasis in a lymph node > 6 cm in greatest dimension and ENE (-); or in a single ipsilateral node > 3 cm in greatest dimension and ENE (+); or multiple ipsilateral, contralateral, or bilateral nodes, any with ENE (+); or a single contralateral node of any size and ENE (+)
N3a	Metastasis in a lymph node > 6 cm in greatest dimension and ENE (-)
N3b	Metastasis in a single ipsilateral node > 3 cm in greatest dimension and ENE (+); or multiple ipsilateral, contralateral, or bilateral nodes, any with ENE (+); or a single contralateral node of any size and ENE (+)
Distant metastases (M)	
M0	No distant metastasis
M1	Distant metastasis

basement membrane [7]. These authors suggested that applying the AJCC 8th edition TNM classification to minor SGCs of the oral cavity may result in overstaging and, consequently, overtreatment [7]. Conversely, Das and Misra emphasized that the submucosal location of minor SGCs facilitates invasion into adjacent structures (e.g., bone, muscle, deep neck spaces), thereby supporting the role of depth of invasion in guiding radical surgical management, with excision extending beyond the greatest depth regardless of basement membrane or mucosal involvement [8]. In the absence of prospective evidence linking depth of invasion to survival in minor SGCs, its applicability as a staging parameter remains unclear. Nonetheless, depth of invasion assessment may aid surgical planning aimed at optimizing locoregional disease control. To address the paucity of evidence regarding the prognostic value of depth of invasion in minor SGCs, imaging-based evaluation – taking into account glandular anatomy and site-specific invasion patterns – may serve as a useful adjunct in preoperative planning [9, 10].

PATHOLOGICAL CLASSIFICATION AND MOLECULAR CHARACTERIZATION: THE IMPORTANCE OF HIGH-GRADE DEDIFFERENTIATION

Recent immunological advances have considerably refined the classification and treatment of both benign and malignant head and neck lesions [11, 12, 13]. Within this context, salivary gland pathology remains one of the most complex areas in head and neck surgical pathology, where novel genetic and molecular insights have driven substantial revisions in the 5th edition of the WHO Classification of Head and Neck Tumors [3, 14]. The key updates in this edition include: 1) the integration of molecular data to define new entities; 2) the incorporation of cytological evaluation in accordance with the Milan system; and 3) the recognition of high-grade transformation as a significant adverse prognostic factor [3, 14].

Given the marked heterogeneity of SGCs, accurate classification is crucial for selecting appropriate treatment strategies and for determining survival and prognostic

outcomes. Notable revisions have been made in the classification of adenocarcinoma, not otherwise specified, which has now been subdivided into microsecretory carcinoma, sclerosing microcystic adenocarcinoma, mucinous adenocarcinoma, salivary carcinoma not otherwise specified, and several emerging entities [3, 14]. In the 5th edition of the WHO classification, molecular alterations were incorporated into the definitions of multiple malignancies, including mucoepidermoid carcinoma, adenoid cystic carcinoma, secretory carcinoma, polymorphous adenocarcinoma, hyalinizing clear cell carcinoma, mucinous adenocarcinoma, and microsecretory adenocarcinoma [3, 14].

Histological grading is considered one of the most important and reliable prognostic factors in SGCs. Grading is based on the cellular and morphological characteristics of individual tumors, as well as the recognition of entity-specific features [15–20]. A high histological grade has been identified as an independent predictor of poor overall survival, increased risk of locoregional recurrence, and higher likelihood of distant metastasis. Brajković et al. reported that perineural invasion was frequently associated with high-grade tumors, correlating with a 32% reduction in overall survival and an 80% increase in the risk of local recurrence [16]. To date, a universal histological grading system for SGCs has not been recommended [3], and evaluation remains particularly challenging in tumors with minimal cellular atypia [3, 14]. In this context, molecular diagnostics play an increasingly important role, providing insights into tumor biology and informing the suitability of patients with recurrent or metastatic disease for targeted therapies.

Furthermore, recent findings suggest that the number of positive lymph nodes, rather than extranodal extension or nodal diameter, may represent a more reliable independent predictor of survival and treatment outcomes in SGCs.

Nodal involvement is widely recognized as one of the most significant prognostic factors influencing survival and treatment outcomes in SGCs [1–4]. In the current 8th edition of the AJCC staging system, the same N-classification is applied to both human papillomavirus-negative squamous cell carcinoma of the upper aerodigestive tract and SGCs, despite the biological and clinical differences between these entities [5]. For squamous cell carcinoma, extranodal extension (ENE) and the largest nodal diameter have been identified as the most critical nodal prognostic indicators, strongly associated with survival and treatment outcomes. However, recent trials reported pitfalls in the AJCC N-staging system for SGCs, including the lack of prognostic significance of ENE, the lack of consideration of parotid lymph nodes, and the significance of bilateral neck metastases, which are extremely rare in SGCs [21–25].

Brajković et al. [16, 24] reported that the number of pathologic lymph nodes, rather than ENE or nodal size, was associated with survival rates and treatment outcomes in SGCs. The prognosis was statistically significantly worse in patients with multiple nodal metastases (pN2 and pN3) than in those with absent or limited nodal involvement (pN0 and pN1) [16, 24]. Lombardi et al. [25] identified both the number and the maximum diameter of nodal metastases as major prognostic determinants of survival. Based on these findings, the authors proposed novel N-classifications stratified according to the number of metastatic nodes (0 *vs*. 1–3 *vs*. ≥ 4) and/or their maximum diameter (< 20 mm *vs*. > 20 mm) [25]. According to Aro et al. [26], the number of positive lymph nodes represents an independent nodal prognostic factor, and patients were stratified into different stages: N0, N1 (1–2 pN+), N2 (3–21 pN+) and N3 (> 22 pN+ or ENE+). Similarly, Lee et al. [27] stratified patients with intermediate- and high-grade SGCs into three nodal stages: N0, N1 (one positive lymph node), and N2 (≥ 2 positive nodes and/or ENE+). In addition, the lymph node ratio, defined as the ratio of positive lymph nodes to the total number of dissected nodes, has been proposed as an independent prognostic factor for survival [28]. Notably, prognostic stratification based on these proposed modifications to pN staging demonstrated superior predictive value compared with the current TNM staging system.

The current AJCC staging system does not recognize parotid lymph nodes as a distinct prognostic category in parotid gland carcinomas (PGCs). However, several studies have highlighted their potential importance. Brajković et al. [24] reported pathologic parotid lymph node involvement in 36% of high-grade PGCs, which was associated with an increased risk of locoregional relapse and lateral neck involvement. Similarly, Lombardi et al. [25] demonstrated that disease localization to parotid lymph nodes significantly increased the risk of cervical nodal metastases and adversely impacted survival. The presence of metastatic parotid nodes in parotidectomy specimens may therefore serve as a predictive marker for cervical metastases in clinically node-negative (cN0) cases, particularly in high-grade tumors. Lim et al. reported a 38% incidence of parotid node metastasis in cN0 cases, which correlated with occult cervical nodal disease and a higher risk of locoregional recurrence [29]. Klussmann et al. [30] further observed that 80% of patients with occult cervical metastases also had involved parotid nodes. Consequently, several authors advocate total parotidectomy as the optimal surgical approach for PGCs, even in early-stage tumors (T1/T2) [31]. In contrast, others have questioned the prognostic significance of parotid lymph nodes, citing the underdeveloped lymphatic network of the gland, which may not reliably harbor metastatic deposits [32]. Taken together, parotid node status may help identify high-risk patients who could benefit from elective neck dissection or adjuvant radiotherapy.

Regional lymph node involvement is widely recognized as an adverse prognostic factor in salivary gland malignancies, underscoring the importance of detecting patients at high risk for regional metastasis. The standard treatment for clinically node-positive (cN+) neck disease consists of neck dissection tailored to the number, size, and extranodal spread of metastatic nodes, followed by adjuvant radiotherapy [4]. In contrast, management of the clinically node-negative (cN0) neck remains controversial and is generally individualized. Elective neck dissection is usually

recommended when the risk of occult nodal metastasis exceeds 15–20% for a given tumor type [33, 34]. Several studies of elective neck dissection in high-grade major SGCs have reported occult metastases in 20–40% of cases [4, 16, 24]. In a large cohort of 2807 patients with adenoid cystic carcinoma of major salivary glands, Xiao et al. [35] demonstrated that elective neck dissection improved overall survival in pT3–pT4 tumors. Similarly, Zbären et al. [36] found that patients with parotid carcinoma who underwent elective neck dissection had a significantly lower rate of locoregional recurrence compared with those managed without elective neck dissection. Occult metastases following surgical resection of SGCs were most frequently identified in cervical levels II, III, and V [37, 38].

DISTANT METASTASES

Distant metastases (DM) represent the leading cause of treatment failure and mortality in patients with SGCs. High-grade histology, advanced TNM stage, and nodal metastases are the principal prognostic factors associated with DM development. Brajković et al. reported a 35% incidence of DM in high-grade SGCs and demonstrated that, among the TNM stage components, pathological nodal status was the independent predictor of both regional and distant metastases as well as poor overall prognosis [39]. Importantly, the number of pathological nodes – but not ENE or nodal size – was significantly associated with the risk of DM. Despite excellent locoregional disease control, the rate of DM remains high, particularly in patients with high-grade tumors. Freitag et al. [15] and Haderlein et al. [40] reported five-year distant metastasis-free survival rates of 62.7% and 56.5%, respectively, in previously treated patients with high-grade SGCs. The treatment of metastatic disease is particularly complicated by tumor heterogeneity.

The National Comprehensive Cancer Network guidelines list multiple management options for patients with distant metastases, including observation, metastasectomy, chemotherapy, concurrent chemoradiotherapy, hormone therapy, and targeted immunotherapy [41, 42]. Selected patients with oligometastatic disease and good performance status may benefit from surgical resection; metastasectomy has been associated with significantly improved five-year survival, reduced overall mortality, and lower cancer-specific mortality compared with non-surgical approaches [41, 43]. Stereotactic body radiation therapy offers a non-invasive alternative for patients with oligometastatic disease who are not candidates for surgery [44, 45]. In contrast, patients with multiple metastases or a high tumor burden are typically managed with systemic therapy, targeted immunotherapy, or observation [41]. In cases of indolent adenoid cystic carcinoma, observation is often appropriate, with treatment initiated only upon disease progression [41, 43]. Conventional platinum-based systemic therapy has demonstrated limited efficacy, providing modest survival benefit while inducing significant systemic toxicity [39, 41, 46].

Table 2. Pitfalls in the current 8th Edition AJCC Staging for Salivary Gland Carcinomas

T	• Prospective trials on the correlation between depth of invasion and survival in minor SGCs; • Significance of tumor grade due to the variable biological and clinical behavior of histological subtypes
N	• Unclear prognostic significance of extranodal extension and nodal size; • No consideration of parotid lymph nodes; • The occurrence of contralateral nodal metastases in major SGCs is extremely uncommon; • Prospective trials on the prognostic significance of major nodal factors (extranodal extension, nodal size, the number of pathological nodes) are needed

Emerging targeted therapies have shown promising results, offering comparable or superior efficacy with lower toxicity relative to conventional chemotherapy [39, 41]. Patients with recurrent or metastatic disease are therefore encouraged to participate in clinical trials. Specific molecular alterations may guide treatment selection, such as RET and NTRK gene fusions, which can be targeted with selpercatinib or pralsetinib (RET inhibitors) and entrectinib or larotrectinib (NTRK inhibitors) [17, 18]. Similarly, androgen receptor (AR) overexpression in salivary duct carcinoma (SDC) may be managed with androgen-deprivation therapy, while human epidermal growth factor receptor 2 (HER2) overexpression or amplification is amenable to trastuzumab and potentially other HER2-targeted agents [19, 20]. In a phase II clinical trial, Takahashi et al. [47] reported a 70.2% response rate in AR-positive and HER2-positive SDC treated with trastuzumab and docetaxel. By contrast, several phase II trials investigating targeted agents such as cetuximab, imatinib, bortezomib, nelfinavir, and dovitinib demonstrated only modest activity in metastatic SGC, with no significant differences in response rates between adenoid cystic carcinoma and non-adenoid cystic carcinoma cases [48].

CONCLUSION

SGCs represent a rare and heterogeneous group of tumors with substantial variability in histological features as well as locoregional and distant metastatic potential. Tumor histological grade, AJCC tumor stage, and nodal status are consistently identified as the most powerful predictors of survival and treatment outcomes. However, further studies are required to clarify the prognostic role of depth of invasion in minor salivary gland carcinomas. Emerging evidence indicates that the current N-classification, particularly the inclusion of ENE, may lack specificity in predicting outcomes for salivary gland malignancies. Given that the primary function of any staging system is to stratify prognosis, the clinical relevance of ENE and nodal dimension in SGC staging warrants further investigation. Recent studies highlight the increasing number of pathologic cervical nodes as a more reliable prognostic factor for survival and treatment outcomes. Additionally, the current AJCC staging system does not account for positive parotid lymph

nodes, despite growing evidence linking their involvement with locoregional treatment failure.

DM remain the leading cause of mortality and treatment failure in SGCs. Overall survival in metastatic disease is generally poor, as only a minority of patients present with operable metastases, while conventional platinum-based systemic therapies confer limited benefit and considerable toxicity. Novel targeted therapies directed at specific molecular alterations have demonstrated encouraging preliminary results; however, these findings remain in the early stages of clinical evaluation (Table 2).

Ethics: The authors affirm that the manuscript has been written in compliance with the ethical standards of the *Serbian Archives of Medicine* and with the institutional ethical regulations applicable to each author.

Conflict of interest: None declared.

REFERENCES

1. Hofmann E, Priebe J, Rieke DT, Doll C, Coordes A, Olze H, et al. Prognostic factors in surgically treated malignant salivary gland tumors. Oral Oncol. 2023;144:106484. [DOI: 10.1016/j.oraloncology.2023.106484] [PMID: 37421674]
2. Vanroose R, Scheerlinck J, Coopman R, Nout E. Clinical outcomes and cost-effectiveness of superficial parotidectomy versus extracapsular dissection of the parotid gland: a single-centre retrospective study of 161 patients. Int J Oral Maxillofac Surg. 2023;52(2):191–8. [DOI: 10.1016/j.ijom.2022.05.001] [PMID: 35624006]
3. Skálová A, Hyrcza MD, Leivo I. Update from the 5th Edition of the World Health Organization Classification of Head and Neck Tumors: Salivary Glands. Head Neck Pathol. 2022;16(1):40–53. [DOI: 10.1007/s12105-022-01420-1] [PMID: 35312980]
4. Brajkovic D, Kiralj A, Ilic M, Vuckovic N, Bijelic B, Fejsa Levakov A. Predictive factors for survival and treatment outcomes of patients with minor salivary gland malignancies: a retrospective study. Eur Arch Otorhinolaryngol. 2023;280(5):2561–74. [DOI: 10.1007/s00405-023-07862-9] [PMID: 36781440]
5. WHO Classification of Tumours Editorial Board. Salivary glands. In: Head and neck tumours. 5th ed. Lyon: International Agency for Research on Cancer; 2024. Available from: https://tumourclassification.iarc.who.int/chaptercontent/52/53
6. Hay AJ, Migliacci J, Karassawa Zanoni D, McGill M, Patel S, Ganly I. Minor salivary gland tumors of the head and neck – Memorial Sloan Kettering experience: incidence and outcomes by site and histological type. Cancer. 2019;125(19):3354–66. [DOI: 10.1002/cncr.32208] [PMID: 31174233]
7. Calabrese L, Nocini R, Spirito F, Gazzini L, Dallari V, Girolami I, et al. The role of the depth of invasion (DOI) in minor salivary glands tumors according to the 8th TNM classification: pitfalls and potential misinterpretations. Oral Oncol. 2022;126:105751. [DOI: 10.1016/j.oraloncology.2022.105751] [PMID: 35144210]
8. Das R, Misra SR. Comment on "The role of the depth of invasion (DOI) in minor salivary glands tumors according to the 8th TNM classification: Pitfalls and potential misinterpretations". Oral Oncol. 2022;127:105819. [DOI: 10.1016/j.oraloncology.2022.105819] [PMID: 35303623]
9. Hiyama T, Kuno H, Sekiya K, Oda S, Kobayashi T. Imaging of malignant minor salivary gland tumors of the head and neck. Radiographics. 2021;41(1):175–91. [DOI: 10.1148/rg.2021200062] [PMID: 33245669]
10. Zheng Y, Xiao Z, Zhang H, She D, Lin X, Lin Y, et al. Differentiation between benign and malignant palatal tumors using conventional MRI: a retrospective analysis of 130 cases. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;125(4):343–50. [DOI: 10.1016/j.oooo.2018.01.006] [PMID: 29477604]
11. Bijelić B, Matić IZ, Besu I, Janković L, Juranić Z, Marušić S, et al. Celiac disease-specific and inflammatory bowel disease-related antibodies in patients with recurrent aphthous stomatitis. Immunobiology. 2019;224(1):75–9. [DOI: 10.1016/j.imbio.2018.10.006] [PMID: 30446336]
12. Obradović R, Vujović S, Desnica J, Stevanović M, Stanišić D, Ognjanović O, et al. Low-level laser efficiency in reparation of bone defects. Srp Arh Celok Lek. 2023;151(1–2):21–5. [DOI: 10.2298/SARH220922003O]
13. Rašković S, Matić IZ, Dorđić M, Damjanović A, Kolundžija B, Grozdanić-Stanisavljević N, et al. Immunoreactivity to food antigens in patients with chronic urticaria. Immunol Invest. 2014;43(5):504–16. [DOI: 10.3109/08820139.2014.892509] [PMID: 24661189]
14. Żurek M, Fus Ł, Niemczyk K, Rzepakowska A. Salivary gland pathologies: evolution in classification and association with unique genetic alterations. Eur Arch Otorhinolaryngol. 2023;280(11):4739–50. [DOI: 10.1007/s00405-023-08110-w] [PMID: 37439929]
15. Freitag V, Lettmaier S, Semrau S, Hecht M, Mantsopoulos K, Müller K, et al. High-grade salivary gland cancer: is surgery followed by radiotherapy an adequate treatment to reach tumor control? Results from a tertiary referral centre focussing on incidence and management of distant metastases. Eur Arch Otorhinolaryngol. 2022;279(5):2553–63. [DOI: 10.1007/s00405-021-07024-9] [PMID: 34436631]
16. Brajkovic D, Kiralj A, Mijatov I, Ilic M. Pathological nodal status as a main predictive factor of survival and treatment outcomes of submandibular salivary gland cancers: a 25-year single center experience. J Stomatol Oral Maxillofac Surg. 2023;124(6):101462. [DOI: 10.1016/j.jormas.2023.101462] [PMID: 37003413]
17. Zhang C, Gu T, Hu Y, Sun J, Xia R, Tian Z, et al. Reevaluation of salivary lymphadenoma: a subgroup identified as Warthin-like mucoepidermoid carcinoma following molecular investigation for MAML2 rearrangement. Arch Pathol Lab Med. 2021;145(6):744–52. [DOI: 10.5858/arpa.2019-0702-OA] [PMID: 32960941]
18. Suzuki K, Harada H, Takeda M, Ohe C, Uemura Y, Kawahara A, et al. Clinicopathological investigation of secretory carcinoma cases including a successful treatment outcome using entrectinib for high-grade transformation: a case report. BMC Med Genomics. 2022;15(1):6. [DOI: 10.1186/s12920-022-01155-6] [PMID: 34991563]
19. Boon E, van Boxtel W, Buter J, Baatenburg de Jong RJ, van Es RJJ, Bel M, et al. Androgen deprivation therapy for androgen receptor-positive advanced salivary duct carcinoma: a nationwide case series of 35 patients in The Netherlands. Head Neck. 2018;40(3):605–13. [DOI: 10.1002/hed.25035] [PMID: 29272069]
20. Locati LD, Cavalieri S, Bergamini C, Resteghini C, Colombo E, Calareso G, et al. Abiraterone acetate in patients with castration-resistant, androgen receptor-expressing salivary gland cancer: a phase II trial. J Clin Oncol. 2021;39(36):4061–8. [DOI: 10.1200/JCO.21.00468] [PMID: 34597119]
21. Cho J, Kim J, Lee JS, Chee CG, Kim Y, Choi SI. Comparison of core needle biopsy and fine-needle aspiration in diagnosis of malignant salivary gland neoplasm: systematic review and meta-analysis. Head Neck. 2020;42(10):3041–50. [DOI: 10.1002/hed.26377] [PMID: 32671867]
22. Cengiz AB, Tansuker HD, Gul R, Emre F, Demirbas T, Oktay MF. Comparison of preoperative diagnostic accuracy of fine-needle aspiration and core needle biopsy in parotid gland neoplasms. Eur Arch Otorhinolaryngol. 2021;278(10):4067–74. [DOI: 10.1007/s00405-021-07022-x] [PMID: 34331572]
23. Eom HJ, Lee JH, Ko MS, Choi YJ, Yoon RG, Cho KJ, et al. Comparison of fine-needle aspiration and core needle biopsy under ultrasonographic guidance for detecting malignancy and for the tissue-specific diagnosis of salivary gland tumors. AJNR Am J Neuroradiol. 2015;36(6):1188–93. [DOI: 10.3174/ajnr.A4247] [PMID: 25678480]
24. Brajkovic D, Kiralj A, Ilic M, Mijatov I, Bijelic B. The number of positive lymph nodes instead of extranodal extension and nodal diameter is an independent predictor of survival and treatment outcome of high-grade parotid gland carcinoma. J Oral Maxillofac Surg. 2024;82(11):1456–74. [DOI: 10.1016/j.joms.2024.06.179] [PMID: 39025128]

25. Lombardi D, Tomasoni M, Paderno A, Mattavelli D, Ferrari M, Battocchio S, et al. The impact of nodal status in major salivary gland carcinoma: a multicenter experience and proposal of a novel N-classification. Oral Oncol. 2021;112:105076. [DOI: 10.1016/j.oraloncology.2020.105076] [PMID: 33137587]
26. Aro K, Ho AS, Luu M, Kim S, Tighiouart M, Clair JMS, et al. Development of a novel salivary gland cancer lymph node staging system. Cancer. 2018;124(15):3171–80. [DOI: 10.1002/cncr.31535] [PMID: 29742277]
27. Lee H, Roh JL, Cho KJ, Choi SH, Nam SY, Kim SY. Positive lymph node number and extranodal extension for predicting recurrence and survival in patients with salivary gland cancer. Head Neck. 2020;42(8):1994–2001. [DOI: 10.1002/hed.26135] [PMID: 32115841]
28. Hong HR, Roh JL, Cho KJ, Choi SH, Nam SY, Kim SY. Prognostic value of lymph node density in high-grade salivary gland cancers. J Surg Oncol. 2015;111(6):784–9. [DOI: 10.1002/jso.23874] [PMID: 25640975]
29. Lim CM, Gilbert MR, Johnson JT, Kim S. Clinical significance of intraparotid lymph node metastasis in primary parotid cancer. Head Neck. 2014;36(11):1634–7. [DOI: 10.1002/hed.23507] [PMID: 24123567]
30. Klussmann JP, Ponert T, Mueller RP, Dienes HP, Guntinas-Lichius O. Patterns of lymph node spread and its influence on outcome in resectable parotid cancer. Eur J Surg Oncol. 2008;34(8):932–7. [DOI: 10.1016/j.ejso.2008.02.004] [PMID: 18358679]
31. Olsen KD, Moore EJ. Deep lobe parotidectomy: clinical rationale in the management of primary and metastatic cancer. Eur Arch Otorhinolaryngol. 2014;271(5):1181–5. [DOI: 10.1007/s00405-013-2616-8] [PMID: 23832259]
32. Varga R, Iro AK, Thimsen V, Sievert M, Agaimy A, Balk M, et al. Locoregional metastatic behavior in a complex mosaic of primary malignant tumors of the parotid gland. Am J Otolaryngol. 2023;44(6):103973. [DOI: 10.1016/j.amjoto.2023.103973] [PMID: 37429129]
33. Westergaard-Nielsen M, Rosenberg T, Gerke O, Dyrvig AK, Godballe C, Bjørndal K. Elective neck dissection in patients with salivary gland carcinoma: a systematic review and meta-analysis. J Oral Pathol Med. 2020;49(7):606–16. [DOI: 10.1111/jop.13034] [PMID: 32430929]
34. Van Herpen C, Vander Poorten V, Skalova A, Terhaard C, Maroldi R, van Engen A, et al. Salivary gland cancer: ESMO–European Reference Network on Rare Adult Solid Cancers (EURACAN) clinical practice guideline for diagnosis, treatment and follow-up. ESMO Open. 2022;7(6):100602. [DOI: 10.1016/j.esmoop.2022.100602] [PMID: 36567082]
35. Xiao R, Sethi RKV, Feng AL, Fontanarosa JB, Deschler DG. The role of elective neck dissection in patients with adenoid cystic carcinoma of the head and neck. Laryngoscope. 2019;129(9):2094–104. [DOI: 10.1002/lary.27814] [PMID: 30667061]
36. Zbären P, Schüpbach J, Nuyens M, Stauffer E. Elective neck dissection versus observation in primary parotid carcinoma. Otolaryngol Head Neck Surg. 2005;132(3):387–91. [DOI: 10.1016/j.otohns.2004.09.029] [PMID: 15746848]
37. Stodulski D, Mikaszewski B, Majewska H, Wiśniewski P, Stankiewicz C. Probability and pattern of occult cervical lymph node metastases in primary parotid carcinoma. Eur Arch Otorhinolaryngol. 2017;274(3):1659–64. [DOI: 10.1007/s00405-016-4407-5] [PMID: 27896425]
38. Lombardi D, Tomasoni M, Lorini L, Gurizzan C, Tomasini D, Ardighieri L, et al. Baseline prognostic factors affecting survival in recurrent and/or metastatic salivary gland adenoid cystic carcinoma. Oral Oncol. 2022;126:105764. [DOI: 10.1016/j.oraloncology.2022.105764] [PMID: 35158286]
39. Brajkovic D, Kiralj A, Ilic M, Mijatov I. Prognostic factors for development of distant metastases in surgically treated high-grade salivary gland carcinomas: results of retrospective single center study with 213 patients. Eur Arch Otorhinolaryngol. 2024;281(9):4947–62. [DOI: 10.1007/s00405-024-08711-z] [PMID: 38709325]
40. Haderlein M, Scherl C, Semrau S, Lettmaier S, Uter W, Neukam FW, et al. High-grade histology as predictor of early distant metastases and decreased disease-free survival in salivary gland cancer irrespective of tumor subtype. Head Neck. 2016;38 Suppl 1:E2041–8. [DOI: 10.1002/hed.24375] [PMID: 26841273]
41. Mimica X, McGill M, Hay A, Karassawa Zanoni D, Shah JP, Wong RJ, et al. Distant metastasis of salivary gland cancer: incidence, management, and outcomes. Cancer. 2020;126(10):2153–62. [DOI: 10.1002/cncr.32792] [PMID: 32097509]
42. National Comprehensive Cancer Network. Head and neck cancers. NCCN Clinical Practice Guidelines in Oncology. Version 2.2022 [Internet]. 2022. Available from: https://www.nccn.org/professionals/physician_gls/pdf/head-and-neck.pdf
43. Shi X, Dong F, Wei W, Song K, Huang N, Lu Z, et al. Prognostic significance and optimal candidates of primary tumor resection in major salivary gland carcinoma patients with distant metastases at initial presentation: a population-based study. Oral Oncol. 2018;78:87–93. [DOI: 10.1016/j.oraloncology.2018.01.009] [PMID: 29496064]
44. Girelli L, Locati L, Galeone C, Scanagatta P, Duranti L, Licitra L, et al. Lung metastasectomy in adenoid cystic cancer: is it worth it? Oral Oncol. 2017;65:114–8. [DOI: 10.1016/j.oraloncology.2016.10.018] [PMID: 28341276]
45. Franzese C, Badalamenti M, Teriaca A, De Virgilio A, Mercante G, Cavina R, et al. Metastasis-directed stereotactic body radiation therapy in the management of oligometastatic head and neck cancer. J Cancer Res Clin Oncol. 2021;147(5):1307–13. [DOI: 10.1007/s00432-021-03518-5] [PMID: 33471186]
46. Yusoh NA, Ahmad H, Vallis KA, Gill MR. Advances in platinum-based cancer therapy: overcoming platinum resistance through rational combinatorial strategies. Med Oncol. 2025;42(7):262. [DOI: 10.1007/s12032-025-02812-3] [PMID: 40518502]
47. Takahashi H, Tada Y, Saotome T, Akazawa K, Ojiri H, Fushimi C, et al. Phase II trial of trastuzumab and docetaxel in patients with human epidermal growth factor receptor 2–positive salivary duct carcinoma. J Clin Oncol. 2019;37(2):125–34. [DOI: 10.1200/JCO.18.00545] [PMID: 30452336]
48. Lorini L, Ardighieri L, Bozzola A, Romani C, Bignotti E, Buglione M, et al. Prognosis and management of recurrent and/or metastatic head and neck adenoid cystic carcinoma. Oral Oncol. 2021;115:105213. [DOI: 10.1016/j.oraloncology.2021.105213] [PMID: 33578204]

Потенцијални недостаци осме ревизије класификације тумора пљувачних жлезда Америчког заједничког комитета за рак

Боривој Бијелић[1], Денис Брајковић[2], Момир Стевановић[3], Владан Ђорђевић[3]

[1]Универзитет у Београду, Стоматолошки факултет, Београд, Србија;
[2]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[3]Универзитет у Крагујевцу, Факултет медицинских наука, Крагујевац, Србија

САЖЕТАК

Карциноми пљувачних жлезда (КПЖ) су ретки малигнитети, који чине мање од 5% свих малигнитета главе и врата. Одликују се значајном разноликошћу хистолошких подтипова, са различитим клиничким и биолошким понашањем. У петој ревизији класификације Светске здравствене организације за болести пљувачних жлезда издваја се 21 тип примарних малигнитета ових жлезда. Преживљавање болесника са КПЖ процењује се коришћењем TNM (тумор, нодус, метастазе) система стадијума болести 8. ревизије Америчког заједничког комитета за рак. Нова истраживања указују на то да тренутни систем одређивања стадијума болести за КПЖ није довољно осетљив да би предвидео исход лечења и преживљавања болесника, посебно у вези са регионалним лимфним метастазама. Наиме, актуелни протокол предлаже исту нодалну класификацију за *HPV*-негативни сквамоцелуларни карцином горњег аеродигестивног тракта и КПЖ, упркос значајним разликама у биолошком понашању, модалитетима лечења и исходима између ова два ентитета. Недостаци у одређивању нодалног статуса код КПЖ укључују нејасан прогностички значај екстранодалног ширења за КПЖ, неодређен статус паротидних лимфних чворова и упитан значај контралатералних метастаза на врату, које су изузетно ретке код КПЖ. Циљ овог наративног прегледног чланка је да укаже на недостатке у актуелној TNM класификацији болести за КПЖ.

Кључне речи: карциноми пљувачних жлезда; стадијум болести; класификација; патолошки нодални статус; дубина инвазије тумора

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарише. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, коментари по позиви, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant – Cross Check* (*iThenticate*). Радови код којих се докаже плагијаризам/ аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (https://www.nlm.nih.gov/mesh/meshhome.html)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.

Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4* (*flv*). У првом кадру филма мора се навести: у

наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике" у СА се објављују фотографије, цртежи, схеме и графикони. Слике се означавају арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).

Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видео-приказа у е-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *https://www.nlm.*

nih.gov/bsd/uniform_requirements.html. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs/en/submission-letter/SubmissionLetterForm2023.pdf*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА И НАКНАДЕ ЗА ОБРАДУ И ОБЈАВЉИВАЊЕ ЧЛАНКА. Да би рад био разматран за објављивање у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 9 Статута Друштва) у години у којој рад предају на разматрање.

Следеће накнаде су обавезне како би рад био прегледан, обрађен и потенцијално објављен у *Српском архиву за целокупно лекарство*:

- накнада за преглед сваког примљеног рада домаћих аутора: 6.000 динара по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада домаћих аутора: 12.000 динара по раду;
- накнада за преглед сваког примљеног рада страних аутора: 75 евра (или 9000 динара) по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада страних аутора: 150 евра (или 18000 динара) по раду.

Накнаде се плаћају пре прегледања, односно пре објављивања рада. Радови за које нису плаћене накнаде неће бити прегледани, односно објављени.

Треба напоменути да уплата накнаде за преглед рада није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и накнаду за преглед чланка, као доказ о уплатама. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за преглед чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Онлајн систем за подношење радова водиће вас кроз поступак уноса података о чланку и отпремања ваших датотека. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и бројеве телефона.

АДРЕСА:
Српско лекарско друштво
Уредништво часописа „Српски архив за целокупно лекарство“
Ул. краљице Наталије 1
11000 Београд
Србија
Телефони: (+381 11) 409-2776, 409-4479
E-mail: *office@srpskiarhiv.rs*
Интернет адреса: *http://www.srpskiarhiv.rs*

ISSN 0370-8179
ISSN Online 2406-0895 OPEN ACCESS

Before submitting their paper to the Editorial Office of the Serbian Archives of Medicine, authors should read the Instructions for Authors, where they will find all the necessary information on writing their manuscript in accordance with the journal's standards. It is essential that authors prepare their manuscript according to established specifications, as failure to do so will result in paper being delayed or rejected. Serbian Archives of Medicine provides no fee for published articles. By submitting a paper for publishing consideration, authors of a paper accepted for publication in the Serbian Archives of Medicine grant and assign all copyrights to the publisher – the Serbian Medical Society.

GENERAL INSTRUCTIONS. *Serbian Archives of Medicine* publishes papers that have not been, either in their entirety or partially, previously published, and that have not been accepted for publication elsewhere. *Serbian Archives of Medicine* publishes papers in English and Serbian. For better availability and citation, authors are encouraged to submit articles of all types in English. The journal publishes the following article types: editorials, original papers, preliminary and short communications, case reports, video-articles, images in clinical medicine, review articles, current topics, articles for practitioners, history of medicine articles, language of medicine articles, medical ethics (clinical ethics, publication ethics) and regulatory standards in medicine, congress and scientific meeting reports, personal view articles, invited commentaries, letters to the editor, book reviews, professional news, In memoriam and other articles. Original papers, case reports, preliminary and short communications, review articles, current topics, video-articles and images in clinical medicine are published in English only, while other article types may be published in Serbian if the Editorial Office reaches such decision.

The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., ^{99}Tc, IL-6, O_2, CD8). If something is commonly written in italics, such as genes (e.g. *BRCA1*), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

CONFLICT OF INTEREST STATEMENT. The manuscript must be accompanied by a disclosure statement from all authors (contained within the Submission Letter) declaring any potential interest or stating that the authors have no conflict of interest. For additional information on different types of conflict of interest, please see World Association of Medical Editors (WAME, *www.wame.org*) policy statement on conflict of interest.

AUTHORSHIP. All individuals listed as authors should be qualified for authorship. Every author should have participated sufficiently in writing the article in order to take responsibility for the whole article and results presented in the text. Authorship is based only on: crucial contribution to the article conception, obtaining of results or analysis and interpretation of results; design of manuscript or its critical review of significant intellectual value; final revision of the manuscript being prepared for publication.

The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized; it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case outline (Outline of cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

KEYWORDS. Below the summary, 3 to 6 keywords or phrases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. *Medical Subject Headings – MeSH* (*https://www.nlm.nih. gov/mesh/meshhome.html*) are to be used for selection of the keywords.

TRANSLATION INTO SERBIAN. The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine," etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

STRUCTURE OF THE MANUSCRIPT. All section headings should be in capital letters using boldface. Original articles and preliminary and short communications should have the following section headings: Introduction (objective is to be stated in the final paragraph of the Introduction), Methods, Results, Discussion, Conclusion, References. A review article and current topic include: Introduction, corresponding section headings, Conclusion, References. The firstly named author of a review article should cite at least five auto-citations (as the author or co-author of the paper) of papers published in peer-reviewed journals. Co-authors, if any, should cite at least one auto-citation of papers also published in peer-reviewed journals. A case report should consist of: Introduction (objective is to be stated in the final paragraph of the Introduction), Case Report, Discussion, References. No names of patients, initials or numbers of medical records, particularly in illustrations, should be mentioned. Case reports cannot have more than five authors. Letters to the editor need to refer to papers published in the *Serbian Archives of Medicine* within previous six months; their form is to be comment, critique, or stating own experiences. Publication of articles unrelated to previously published papers will be permitted only when the journal's Editorial Office finds it beneficial.

All enclosures (tables, graphs, photographs, etc.) should be placed at the end of the manuscript, while in the body of the text a particular enclosure should only be mentioned and its preferred place indicated. The final arrangement (position) of the enclosures will depend on page layout.

ABBREVIATIONS. To be used only if appropriate, for very long names of chemical compounds, or as well-known abbreviations (standard abbreviations such as DNA, AIDS, HIV, ATP, etc.). Full meaning of each abbreviation should be indicated when it is first mentioned in the text unless it is a standard unit of measure. No abbreviations are allowed in the title. Abbreviations in the summary should be avoided, but if they have to be used, each of them should be explained when first mentioned in the text of the paper.

DECIMAL NUMBERS. In papers written in English, including text of the manuscript and all enclosures, a decimal point should be used in decimal numbers (e.g. 12.5 ± 3.8), while in Serbian papers a decimal comma should be used (e.g. 12,5 ± 3,8). Wherever applicable, a number should be rounded up to one decimal place.

UNITS OF MEASURE. Length, height, weight and volume should be expressed in metric units (meter – m, kilogram – kg, gram – g, liter – l) or subunits. Temperature should be in Celsius degrees (°C), quantity of substance in moles (mol), and blood pressure in millimeters of mercury column (mmHg). All results of hematological, clinical and biochemical measurements should be expressed in the metric system according to the International System of Units (SI units).

LENGTH OF PAPER. The entire text of the manuscript – title page, summary, the whole text, list of references, all enclosures including captions and legends (tables, photographs, graphs, schemes, sketches), title page and summary in Serbian – must not exceed 5,000 words for original articles, review articles and articles on history of medicine, and 3,000 words for case reports, preliminary and short communications, current topics, articles for practitioners, educational articles and articles for "Language of medicine", congress and scientific meeting reports; for any other section maximum is 1,500 words.

Video-articles are to last 5–7 minutes and need to be submitted in the flv video format. The first shot of the video must contain the following: title of the journal in the heading (*Serbian Archives of Medicine*), title of the work, last names and initials of first and middle names of the paper's authors (not those of the creators of the video), year of creation. The second shot must show summary of the paper, up to 350 words long. The final shot of the video may list technical staff (director, cameraman, lighting, sound, photography, etc.). Video-articles need to be submitted along with a separate summary (up to 350 words), a single still/ photograph as an illustration of the video, and a statement signed by the technical staff renouncing copyrights in favor of the paper's authors. To check the required number of words in the manuscript, please use the menu *Tools–Word Count*, or *File–Properties–Statistics*.

ARTICLE ENCLOSURES are tables, figures (photographs, schemes, sketches, graphs) and video-enclosures.

TABLES. Each table, with its legend, should be self-explanatory. The title should be typed above the table and any explanatory information under the table. Tables should be numbered in Arabic numerals in order of citation in the text. Use *MS Word*, the menu *Table–Insert–Table*, inserting the adequate number of rows and columns. By the right click of the mouse, use the options *Merge Cells* and *Split Cells*. Use *Times New Roman*, font size 12 pt, with single line spacing and no indent to draw tables. Abbreviations used in tables should be explained in the legend below each respective table.

If the manuscript is entirely in the Serbian language, tables and corresponding legend should be both in Serbian and English. Also, the table cells should contain text in both languages (do not create two separate tables with a single language!).

FIGURES. Figures are all types of visual enclosures, and photographs, schemes, sketches and graphs are published as 'figures' in the *Serbian Archives of Medicine.* Figures should be numbered in Arabic numerals in order of citation in the text. Only original digital photographs (black-and-white or color), of minimum 300 dpi, and *jpg* or *tiff* format, are acceptable (small, blurry and photographs of poor quality will not be accepted for publishing!). If authors do not possess or are not able to provide digital photographs, then the original photos should be scanned in 300 dpi, and saved in original size. If a paper needs to be illustrated with a considerable number of figures, several figures will be published within the paper, and the rest will be available in the electronic version of the paper as a PowerPoint presentation (every figure needs to be numbered and be accompanied by legend). Video-enclosures (illustrations of a paper) can last 1–3 minutes and are submitted in the *flv* format. Along with the video, a still/photograph representative of the video is also needed, as it will be used as a placeholder in the electronic version of the paper, and as an illustration in the printed version.

If the manuscript is entirely in the Serbian language, photographs and corresponding legend should be both in Serbian and English.

Photographs may be printed and published in color, but possible additional expenses are to be covered by the authors.

GRAPHS. Graphs should be plotted in *Excel* in order to see the respective values distributed in the cells. The same graphs should be copied and pasted to the *Word* document, numbered in Arabic numerals by order of citation in the text. The text in the graphs should be typed in *Times New Roman*. Abbreviations used in graphs should be explained in the legend below the respective graph. In the printed versions of papers, graphs are generally published in black-and-white; therefore, it is suggested to avoid the use of colors in graphs, or to utilize colors of significant difference in brightness.

If the manuscript is entirely in the Serbian language, graphs and corresponding legend should be both in Serbian and English.

SCHEMES (SKETCHES). Schemes and sketches are to be submitted in *jpg* or *tiff* format. Schemes should be drawn in *CorelDraw* or *Adobe Illustrator* (programs for drawing vectors, curves, etc.). The text in the schemes should be typed in *Times New Roman*, font size 10 pt. Abbreviations used in schemes should be explained in the legend below the respective scheme. If the manuscript is entirely in the Serbian language, schemes and corresponding legend should be both in Serbian and English.

ACKNOWLEDGMENT. List all those individuals having contributed to preparation of the article but having not met the criteria of authorship, such as individuals providing technical assistance, assistance in writing the paper or running the department securing general support. Financial aid and all other support in the form of sponsorship, grants, donations of equipment and medications, etc., should be mentioned as well.

REFERENCES. The reference list is the responsibility of the authors. Cited articles should be readily accessible to the journal's readers. Therefore, following each reference, its DOI number and PMID number (if the article is indexed for MEDLINE/PubMed) should be typed. References should be numbered in Arabic numerals in order of citation in the text. The overall number of references should not exceed 30, except in review articles, where maximum of 50 is acceptable, and in meta-analyses, where up to 100 references are allowed. The number of citations of original articles must be at least 80% of the total number of references, and the number of citations of books, chapters and literature reviews less than 20%. If monographs and articles written by Serbian authors could be included in the reference list, the authors are obliged to cite them. The majority of the cited articles should not be older than five years. Use of abstracts as references is not allowed. If it is important to comment on results published solely in the form of an abstract, it is necessary to do so within the text of the article. The references of articles accepted for publication should be designated as *in press* with the enclosed proof of approval for publication.

The references are cited according to the Vancouver style (*Uniformed Requirements for Manuscripts Submitted to Biomedical*

Journals), rules and formats established by the International Committee of Medical Journal Editors (*http://www.icmje.org*), used by *the U.S. National Library of Medicine* and scientific publications databases. Examples of citing publications (journal articles, books and other monographs, electronic, unpublished and other published material) can be found on the web site *https://www.nlm.nih. gov/bsd/uniform_requirements.html*. In citation of references, the defined standards should be strictly followed, because it is one of the essential factors of indexing for classification of scientific journals.

SUBMISSION LETTER. The manuscript must be accompanied by the Submission Letter, which is signed by all authors and includes the following: 1) statement that the paper has never been published and concurrently submitted for publication to any other journal; 2) statement that the manuscript has been read and approved by all authors who have met the criteria of authorship; and 3) contact information of all authors of the article (address, email, telephone number, etc.). Blank Submission Letter form can be downloaded from the journal's web site (*http://www.srpskiarhiv.rs/en/submission-letter/SubmissionLetterForm2023.pdf*).

Additionally, the authors should submit the following copies of all permits for: reproduction of formerly published material, use of illustrations and publication of information on known people or disclosure of the names of people having contributed to the work.

MEMBERSHIP FEE AND REVIEW AND PUBLICATION FEES. For an article to be considered for publishing in the *Serbian Archives of Medicine*, all its authors and co-authors residing in Serbia (if they are medical doctors or doctors of dental medicine) must be members of the Serbian Medical Society (in accordance with Article 9 of the Society's Bylaw) for the year in which the paper is being submitted.

The following fees are required for the review, processing, and publication of manuscripts in the *Serbian Archives of Medicine*:

- Review fee for each manuscript submitted by domestic authors: 6,000 dinars per manuscript;
- Publication fee for each accepted manuscript by domestic authors: 12,000 dinars per manuscript;
- Review fee for each manuscript submitted by foreign authors: 75 EUR (or 9000 dinars);
- Publication fee for each accepted manuscript by foreign authors: 150 EUR (or 18000 dinars)

Respective fees must be paid before a manuscript is reviewed or published. Manuscripts for which the prescribed fees have not been paid will not be processed further.

Please note that the payment of this charge does not guarantee acceptance of the manuscript for publication and does not influence the outcome of the review procedure, in accordance with the Good Publication Practice. The journal accepts donations from sponsors to create a sum for payment reductions or waivers for authors unable to cover the article processing and publication charges (a justification of the inability to pay should be provided in such cases). Institutions (legal entities) cannot by their subscription cover this condition on behalf of the authors (natural persons). Copies of deposit slips for membership and article processing charge should be enclosed with the manuscript. Foreign authors are under no obligation to be members of the Serbian Medical Society. All the relevant information can be obtained via email address of the Editorial Office (*office@srpskiarhiv.rs*) and on the journal's web site (*http://srpskiarhiv.rs/en/subscription/*).

SUBMISSION. The online submission system will guide you through the process of entering your article details and uploading your files. All correspondence, including notification of the Editorial Office, requests for revision and editors' decision will be sent by e-mail.
Please submit your manuscript and all enclosures via: ***http://www.srpskiarhiv.rs***.

NOTE. The papers not complying with these instructions will not be reviewed and will be returned to the authors for revision. Observing the instructions for preparation of papers for the *Serbian Archives of Medicine* will shorten the time of the entire process of publication and will have a positive effect on the quality and timely release of the journal's issues.
For further information, please contact us via the following address:

ADDRESS:
Serbian Archives of Medicine Editorial Office
Kraljice Natalije 1
11000 Belgrade
Serbia
Phones: (+381 11) 409-2776, 409-4479
E-mail: *office@srpskiarhiv.rs*
Website: *www.srpskiarhiv.rs*
ISSN 0370-8179
ISSN Online 2406-0895 OPEN ACCESS

CIP – Каталогизација у публикацији
Народна библиотека Србије, Београд

61(497.11)

СРПСКИ архив за целокупно лекарство : званичан часопис Српског лекарског друштва = Serbian Archives of Medicine : official journal of the Serbian Medical Society / главни и одговорни уредник Гордана Теофиловски-Парапид. - Књ. 1 (1874)-књ. 2 (1875) ; књ. 3 (1879)- књ. 8 (1881) ; књ. 9 (1887)-књ. 10 (1888) ; књ. 11 (1894)-књ. 12 (1895) ; год. 1, бр. 1/2 (1895)- . - Београд : Српско лекарско друштво, 1874-1875; 1879-1881; 1887-1888; 1894-1895; 1895-(Београд : Службени гласник). - 29 cm

Двомесечно. - Текст на енгл. језику. - Има суплемент или прилог: Српски архив за целокупно лекарство. Суплемент = ISSN 0354-2793. - Друго издање на другом медијуму: Српски архив за целокупно лекарство (Online) = ISSN 2406-0895
ISSN 0370-8179 = Српски архив за целокупно лекарство
COBISS.SR-ID 3378434

Nimulid®

nimesulid

Amicus
a Swixx BioPharma company

PM-RS-2023-5-2231, maj 2023

The Journal Serbian Archives of Medicine is indexed in: Science Citation Index Expanded, Journal Reports/Science Edition, Web of Science, Scopus, EBSCO, Directory of Open Access Journal, DOI Serbia

CONTENTS