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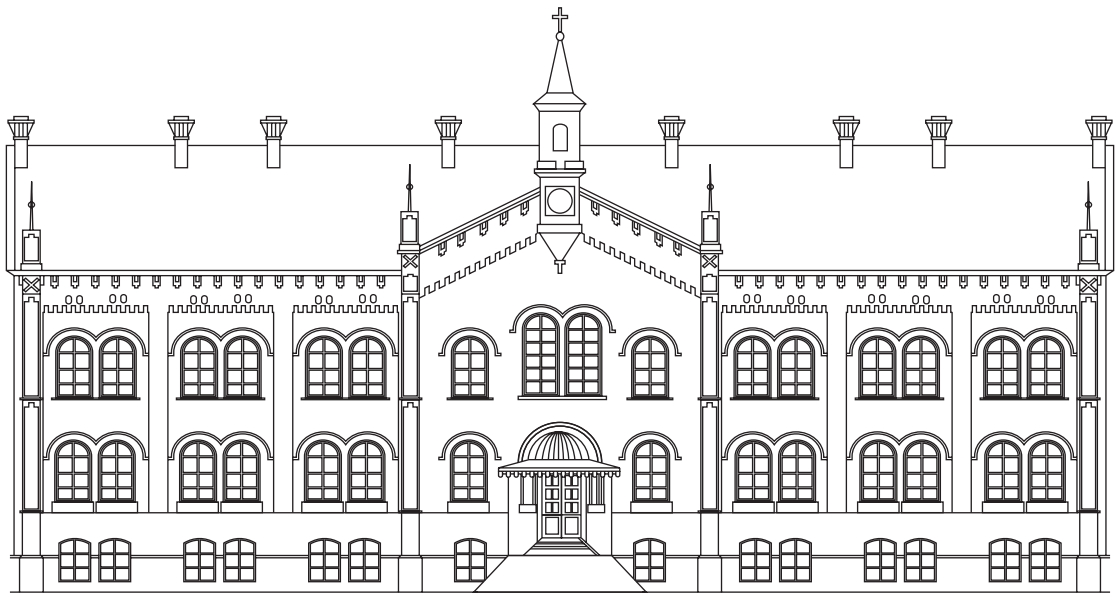

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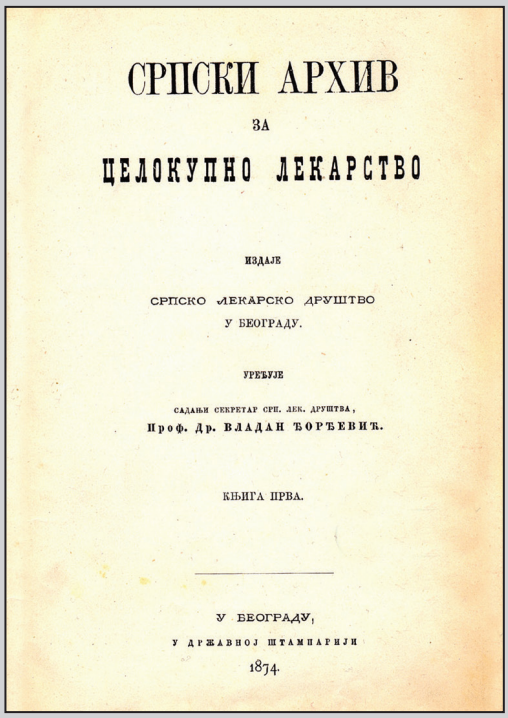

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ЗА

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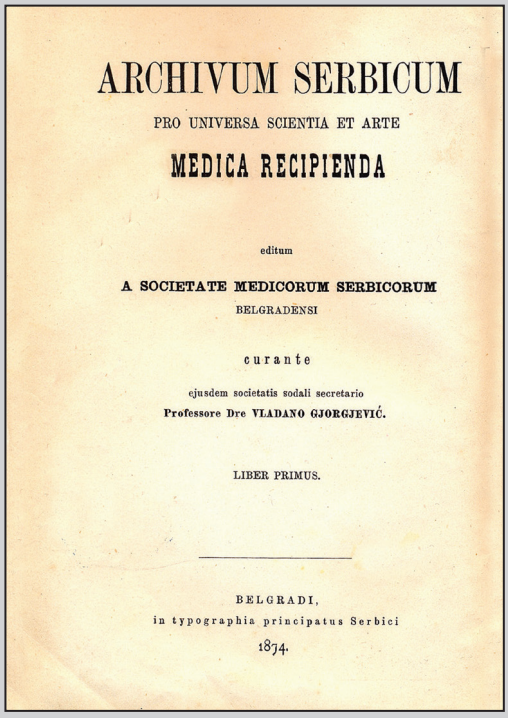

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The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparative evaluation of clinical diagnostic trials for pulpal status assessment and establishment of reference values in healthy young adults

Ema Krdžović Lazić[1], Aleksandar Jakovljević[2,3], Marija S. Milić[4], Jugoslav Ilić[1], Nenad Nedeljković[5], Katarina Beljić-Ivanović[1]

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SUMMARY

Introduction/Objective Traditional diagnostic methods for assessing pulp vitality are commonly used but lack precision and reliability, whereas recent advancements offer non-invasive alternatives, focusing on pulp tissue blood flow and oxygen saturation. This study was designed to evaluate and compare the reliability of all available clinical diagnostic methods for assessing pulp status and to establish reference values in healthy young individuals.

Methods This cross-sectional observational study enrolled 25 voluntary participants (27.8 ± 5.2 years old), with 150 upper front teeth. The participants were healthy individuals, non-smokers, with teeth having a mature apex, no or minimal restorations, being periodontally healthy, and with radiographically visible pulp chambers. Various diagnostic tests were conducted, including electric (EPT), cold (CPT), and hot pulp testing (HPT), laser Doppler flowmetry (LDF), and pulse oximetry (PO); the reference values were established.

Results Statistically significant differences were observed among all tooth groups (central incisors, lateral incisors, and canines) for EPT, LDF, and PO. In contrast, CPT and HPT showed no statistically significant differences between the tooth groups. The mean values across all patients were as follows: EPT – 17.7 ± 5.4, PO – 80.6 ± 1.8%, and LDF – 4.6 ± 1.6 perfusion units. Correlation analysis showed no significant relationships between the tests.

Conclusion Modern diagnostic techniques show promise in offering more reliable results. Establishing reference values is essential for improving diagnostic accuracy. These values will not only enhance clinical decision-making but also serve as a foundation for future investigations.

Keywords: clinical diagnostic methods; laser Doppler flowmetry; pulse oximetry; electric pulp testing; dental pulp

INTRODUCTION

Dental caries remains one of the most prevalent diseases in the orofacial region and significantly contributes to dental pulp disease [1]. Pulpitis, the inflammation of the pulp tissue, is a common sequela of dental caries and can lead to significant discomfort in affected individuals [2]. Although there is growing interest in the need for improved classification and understanding of pulp tissue health [3] pulpitis is currently categorized as reversible or irreversible, depending on the extent of tissue damage [4].

Reversible pulpitis involves initial inflammation that can heal and restore. Patients typically have mild to moderate, localized pain triggered by stimuli, though some may experience no pain, diagnosed by subjective or objective complaint [4]. In contrast, irreversible pulpitis is marked by permanent pulp damage. Acute irreversible pulpitis presents with rapid-onset, intense pain, often spontaneous and hard to localize, with possible heat sensitivity, swelling, and chewing difficulties. Chronic irreversible pulpitis progresses slowly with dull, recurrent pain, usually triggered by hot or cold stimuli [4].

Establishing an adequate diagnosis is one of the keys to successful therapy. However, the lack of objective criteria that indicate the actual state of the pulp tissue presents a challenge in making adequate decisions during treatment [5]. Historically, there has been a tendency to opt for vital pulp extirpation, which has contributed to the relatively low success rates of endodontic procedures and the increased need for retreatment [6].

To address this issue, the European Society of Endodontology initiated a project called "S3 Clinical Guidelines," aimed at developing evidence-based guidelines for the diagnosis and treatment of pulp and periapical diseases [7]. As part of this project, Donnermeyer et al. [8]

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conducted a systematic review that evaluated the effectiveness of diagnosing pulp diseases using various diagnostic tests [7]. The authors concluded that the existing scientific knowledge provides a very low level of evidence on the accuracy and reproducibility of the current diagnostic tests and a lack of reliable prognostic indicators that enable a reliable preoperative assessment of the outcome of vital pulp treatment. This further complicates the diagnostic process and makes it difficult to predict the success of therapy [8]. Before any test, meticulous and detailed anamnesis is conducted to reveal pain history, suggesting a potential inflammatory state of the pulp. The most frequently used diagnostic tools for evaluating the state of the pulp are sensitivity tests [electric pulp testing (EPT), cold pulp testing (CPT), and heat pulp testing (HPT)] [9]. Despite the widespread use, their lack of precision and reproducibility represent their main limitations, primarily due to the subjective response of the patient, the interpretation of the dentist, and the inability to detect blood flow in the pulp [10, 11]. In order to overcome these limitations, modern diagnostic tools, laser Doppler blood flowmeters (LDF) and pulse oximeters (PO), were introduced into practice. These methods are based on the assessment of the blood flow in the pulp and its oxygen saturation without relying on the subjective responses of the patients and the personal interpretation of the dentist [12–15].

While many studies have assessed individual diagnostic tools for pulp tissue [8], none have simultaneously compared all conventional and modern methods or established reference values in healthy young individuals.

This study aimed to compare the reliability of all clinical diagnostic methods (EPT, CPT, HPT, LDF, PO) for pulpal assessment and to establish reference values in healthy young individuals for future correlation with pulp-derived laboratory tests.

METHODS

This observational cross-sectional study was conducted at the Department of Restorative Odontology and Endodontics, School of Dental Medicine, University of Belgrade, Serbia. All data were collected from January to September 2024. Prior to any testing, all participants fulfilling the inclusion criteria were informed about the study's objectives and procedures, and they provided written informed consent. This investigation enrolled 25 voluntary participants (27.8 ± 5.2 years old, 15 females and 10 males) comprising a total of 150 upper front teeth. The sample size was calculated using a power analysis to ensure sufficient statistical power for detecting significant differences. The calculation was based on a previous pilot study and the variability of the key measures.

Participants met the following inclusion criteria: i) healthy individuals (ASA I), ii) non-smokers, iii) mature apex, iv) no or minimal restoration on upper front teeth, v) periodontally healthy, and vi) radiographically visible coronal pulp chamber. Exclusion criteria included: i) presence of any systemic disease, ii) smokers, iii) caries or massive restorations, iv) discolorations of the tooth, v) radiographically invisible coronal pulp chamber, vi) radiographical signs of periapical lesion and vii) orthodontically treated teeth.

Patients were instructed to abstain from foods and beverages for at least two hours before attending. All measurements were performed in a temperature-controlled room using the same unit and keeping the patient's head in the same position, resting for 10 min in the unit before the measurements.

Each patient underwent five pulp tests. EPT, CPT, HPT, and PO were performed with 15-minute intervals. Tests were repeated twice by the same operator (EKL) to reduce bias, and results were presented as mean values. Any unclear responses prompted retesting.

For CPT, a No. 2 cotton pellet with a refrigerant spray (1,1,1,2-tetrafluoroethane) [Cold Spray (ROEKO Endo-Frost); Coltene Whaledent, Cuyahoga Falls, OH, USA] was used and placed onto the middle third of the buccal surface for 18 seconds or until the participant raises a hand to indicate a cold sensation, with the standardized temperature of -26°C [16].

As for the HPT, a heated gutta-percha rod was placed on the middle third of the buccal tooth surface for 18 seconds or until the participant raised a hand to indicate a hot sensation, with a standardized temperature of 80°C [16].

EPT was performed using a digital Pulp Tester (Analytic Technology Pulp Tester; Analytic Technology, Redmond, WA, USA). The tooth was air-dried and isolated with cotton rolls to prevent false responses from surrounding tissues. The lip clip was attached to the lip, and the electrode was placed on the middle third of the buccal surface. A slow increase in the current, calibrated at medium speed, permitted patients to report any pain sensation. The detected stimulus threshold was recorded as a positive response, and the exact value was noted [16, 17].

LDF is a semi-quantitative, non–invasive method used for the assessment of pulpal blood flow (PBF). Red light with a wavelength of 632.8 nm was produced by a 1 mW helium–neon laser diode within the flowmeter (PeriFlux System PF 5001, Perimed, Jarfalla, Sweden) and transmitted to the tooth surface along the fiber-optic conductor inside a round probe (407-2, Perimed) with a cross-sectional diameter of 1 mm. The probe simultaneously received the reflected and scattered light via afferent optical fiber, which was then registered by a photodetector in the flowmeter. According to the Doppler effect, light reflected from moving red blood cells shifts in frequency, allowing calculation of their concentration and velocity. This shift indicates tissue blood flow, expressed in semi-quantitative perfusion units (PU). Before data collection, a colloidal suspension of latex particles (Perimed Motility Standard) was used to calibrate the flowmeter on the wide band to a specific value of 250 PU. The artifact filter was activated with a sampling frequency set at 32 Hz. PBF values were stored on a computer software (Perisoft, Perimed) for further analysis. To stabilize and obtain a reproducible position of the probe on the tooth surface, custom-made splints were prepared (Zhermack dental Hydrorise putty and regular body impression material, Faclon Medical impression trays, Vacum former EV2 3A Medes, and Gasket for splint 080 2mm 1/12), and used

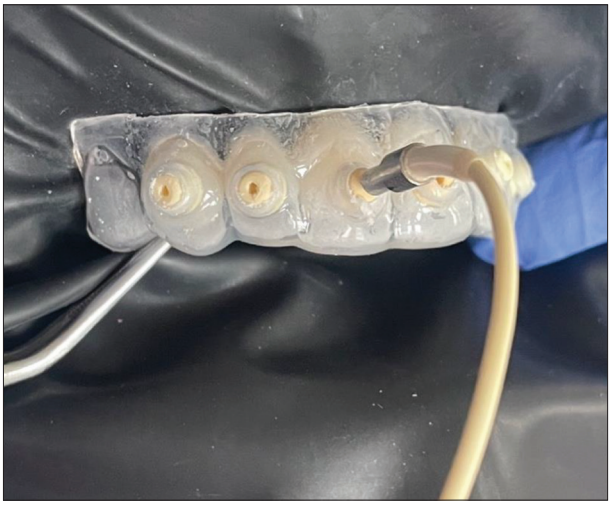

Figure 1. Laser Doppler flowmetry probe holder

Figure 2. Custom-made pulse oximeter dental probe

to secure the position of probe holder (PH07-6, Perimed). Aperture for the probe holder was positioned labially on the cervical third of the tooth crown, at least 1 mm from the gingival margin (Figure 1). Measurements were repeated three times with a 1-minute pause, and the mean value was obtained as a final result, all performed in a quiet room with a constant ambient temperature [13, 14, 18].

For PO testing, the fifth-generation Nellcor OxiMax 550 pulse oximeter (Tyco Healthcare Group LP) was used. The OxiMax system's "sensor message" function analyzes data from the sensor, using a proprietary algorithm that interprets parameters stored on the sensor's memory chip and evaluates real-time signal characteristics from the patient. A Nellcor OxiMax™ Dura–Y D-YS multisite oxygen sensor (Tyco Healthcare Group LP) was chosen due to its smaller dimensions, fitting better to the mesio-distal width of human permanent teeth. To ensure consistent and accurate sensor placement, a custom-designed pulse oximeter sensor holder was developed, designated as the pulse oximeter dental probe (Figure 2). It was positioned on each tooth, aligning so that the sensor's light beam traveled from the buccal to lingual surfaces through the middle third of the tooth crown. After 30 seconds of monitoring each tooth, values were recorded. An oxygen saturation reading within the range of 75–90% indicated a positive response, while any value below 75% indicated a negative response [11, 12, 19].

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA). Data were expressed as means ± standard deviations for continuous variables and as frequencies and percentages for categorical data. The normality of data distribution was assessed using the Shapiro–Wilk test. For normally distributed data, one-way analysis of variance (ANOVA) with Bonferroni post hoc correction was used to compare mean values across the three tooth groups. Non-normally distributed variables were analyzed using the Kruskal–Wallis test, and pairwise comparisons were performed using the Mann–Whitney U test with Bonferroni adjustment (corrected significance threshold: $p < 0.016$). Categorical variables were compared using the χ^2 test. Pearson correlation coefficients were calculated to assess relationships between diagnostic test parameters (EPT, PO, and LDF), with statistical significance set at $p < 0.05$.

Ethics: This study was approved by the local ethics committee (protocol number: 36/36).

RESULTS

The mean values of the investigated parameters for each group of teeth are presented in Figure 3. All teeth responded positively to CPT and HPT. When analyzing the mean values across all patients for each of the three tests (EPT, LDF, and PO), distinct patterns emerged. For EPT, the average threshold was 17.7 ± 5.4, indicating a typical level of electrical stimulus required to provoke a sensory response from the pulp. This reflects the overall excitability of pulpal nerve fibers across the sample. For PO, the mean oxygen saturation value was 80.6 ± 1.8%, suggesting consistent readings of pulpal blood oxygen levels among participants. Regarding LDF, the mean value was 4.6 ± 1.6 PU, representing the average pulpal blood flow. This measure reflects microcirculatory activity within the pulp and serves as an important indicator of tissue vitality. Together, these mean values provide a reliable baseline profile for pulp sensitivity, oxygen saturation, and vascular perfusion, forming the basis for comparisons between individual tooth groups. Statistically significant differences were observed between the three investigated tooth groups (central incisors, lateral incisors, and canines) for all parameters, except for cold and hot pain thresholds.

To identify which tooth group differed significantly, we performed multiple pairwise comparisons. To account for the increased risk of Type I errors due to multiple testing, we

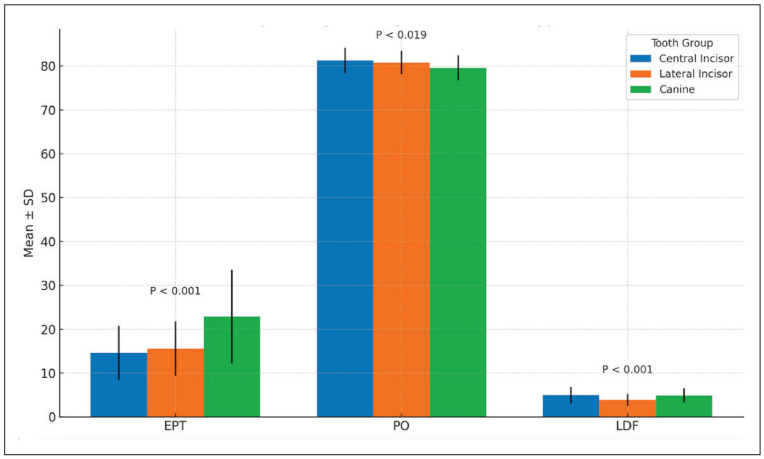

Figure 3. The mean values of the investigated parameters for each tooth group;

SD – standard deviation; EPT – electric pulp testing; PO – pulse oximetry; LDF – laser Doppler flowmetry

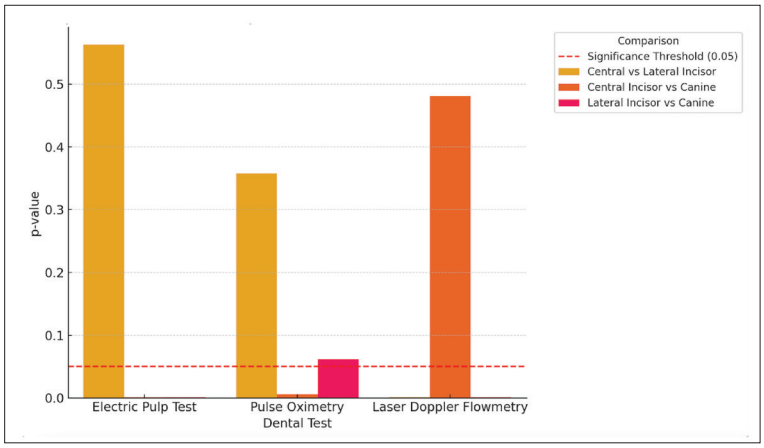

Figure 4. Multiple comparisons between tooth groups (least significant difference test) for electric pulp test, pulse oximetry, and laser doppler flowmetry

DISCUSSION

Achieving an accurate assessment of dental pulp conditions poses a considerable challenge in clinical practice, underscoring the need for improved tools to enhance diagnostic precision. To our knowledge, this is the first study to assess and compare all available clinical diagnostic tests for testing pulp vitality within the same research [20]. The analysis of the mean values across different tooth groups revealed distinct patterns in EPT, PO, and LDF, while all teeth showed positive responses to CPT and HPT.

Pulp sensitivity tests (EPT, CPT, HPT) assess the nerve response of the pulp rather than its blood flow [20]. Because nerve tissue is highly resistant to inflammation, it may still respond even when surrounding tissues have been degraded, leading to potential false-positive results [19]. Our EPT results showed variability among the tooth groups, with canines requiring significantly higher stimulation than central and lateral incisors, likely due to the greater pulp chamber size [21].

To ensure stable placement of the PO sensor on each tooth, a specialized dental pulse oximeter holder was designed and fabricated based on the model by Gopikrishna et al. [12]. It maintained a consistent alignment between the sensor components and the tooth, ensuring a fixed path length for the light emitted by the LED sensor and received by the photoreceptor sensor, allowing for accurate readings. The relatively low standard deviation indicates limited variability, pointing to a stable physiological range in pulpal oxygenation for the tested teeth. On the other side, the obtained reference values differ from those reported by Calil et al. [22], due to their use of signal amplification 2.5 times higher than standard levels.

Previous research indicated that LDF is among the most reliable methods for detecting pulp vitality, although its routine application revealed technical challenges that must be considered (patient head movement, interference, and the need for custom-made probes) [20]. These may also lead to inaccurate representations of pulp health in cases where the pulp is diseased yet maintains a viable blood supply [23]. The heterogeneity of blood flow within the dental pulp [24] may further complicate the acquisition of consistent measurements. In this study, all blood flow measurements were standardized by positioning the probe at least 1 mm away from the gingiva, isolating the area with a black rubber dam, and placing the probe within a custom-made splint. This setup aimed to enhance the accuracy and consistency of the readings by minimizing movement and isolating the measured area. Our results are consistent with those obtained using the same type of probe by Ingolfsson et al. [14]. However, significant differences

applied the Bonferroni correction by dividing the standard p-value threshold (0.05) by the number of comparisons, which was 3. Consequently, a difference was considered statistically significant at $p < 0.016$. Significant differences were found between the canine and both incisors in EPT ($p < 0.001$), with no difference between the central and lateral incisors ($p = 0.563$). In PO, only the central incisor and canine differed significantly ($p = 0.006$). For LDF, significant differences were seen between the central and lateral incisors and between the lateral incisor and canine (both $p < 0.001$), indicating distinct pulpal blood flow in the lateral incisor. All pairwise comparison results for the investigated tests (EPT, PO, and LDF) are presented in Figure 4. The correlation analysis revealed no significant relationships between the EPT, PO, and LDF. Specifically, the Pearson correlation between the EPT and PO was -0.067 ($p = 0.457$), indicating no significant association. Similarly, no significant correlation was found between the EPT and LDF ($r = 0.013$, $p = 0.891$) or between PO and LDF ($r = 0.029$, $p = 0.751$). The only statistically significant correlation observed in the analysis was between LDF and Doppler flow variability, which indicates that higher flow is associated with greater variability in flow.

between the central and lateral incisors, as noted by Seasano et al. [25], can be attributed to the thicker dentin, which impedes deep photon penetration and direct transmission to the receiving fiber.

A key factor contributing to the differences between the results of this and previous studies may lie in variations in research procedures and the control of influencing factors, such as sample size. Dastmalchi et al. [20] examined 24 pairs of teeth, Gopikrishna et al. [12] examined 80 teeth, Karayilmaz and Kirzioğlu [13] examined 59 pairs, and Janani et al. [19] assessed only 29 teeth. On the other hand, in our study, 150 teeth (75 pairs) have been examined, which may account for some of the observed differences compared to the previous investigations.

Gopikrishna et al. [10] studied patients aged 15–40 years, Calil et al. [22] examined individuals 26–38 years old, Karayilmaz and Kirzioğlu [13] focused on those aged 12–18 years, and Dastmalchi et al. [20] examined individuals 18–50 years old. Our study included patients aged 20–40 years, which is in accordance with most of the previously mentioned studies.

The main strengths of this study include the use of a single, calibrated operator, as well as the implementation of duplicate measurements for all tests to minimize measurement errors. All available clinical diagnostic tests were performed on the same tooth, allowing for a comprehensive assessment. The sample size was determined based on a pilot study conducted within the same experimental framework.

A limitation of this study may be the restricted selection of tooth types. Including all tooth types could improve the study, but may complicate diagnosis due to multi-root variability in diagnosis. Future research should place greater emphasis on molecular analyses of inflammatory mediators, which may serve as predictive markers for different stages of pulpal pathology [26, 27, 28].

Establishing reference values is essential for improving diagnostic accuracy and supporting clinical decision-making, serving as a foundation for future investigations. Before initiating any kind of dental treatment, a clinician should collect all information on pulp disease, combining this with clinical examination and results from all available pulp tests. With recent scientific advancements and with the integration of new technologies into dentistry, the endodontics field has had great benefit from updated diagnostic tools, such as PO and LDF, which should ideally become standard in everyday practice.

CONCLUSION

Modern diagnostic methods hold promise in delivering results that are both more objective and reliable. Establishing reference values is essential for improving diagnostic accuracy. These values will not only enhance clinical decision-making but also serve as a foundation for future investigations. While EPT remains a common tool in clinical practice, the incorporation of PO into routine diagnostic procedures represents a significant step forward. However, future research should focus on emerging diagnostic methods, including laboratory analyses and the evaluation of inflammatory mediator levels, to further enhance diagnostic accuracy.

Conflict of interest: None declared.

REFERENCES

1. Wen PYF, Chen MX, Zhong YJ, Dong QQ, Wong HM. Global burden and inequality of dental caries, 1990 to 2019. J Dent Res. 2022;101(4):392–9. [DOI: 10.1177/00220345211056247] [PMID: 34852668]
2. Väisänen M, Siukosaari P, Tjäderhane L. How epigenetics and miRNA affect gene expression in dental-pulp inflammation: a narrative review. Int Endod J. 2025;58(6):833–47. [DOI: 10.1111/iej.14211] [PMID: 40016884]
3. Abbott PV. Pulp, root canal and peri-radicular conditions: the need for re-classification. Iran Endod J. 2024;19(3):158–75. [DOI: 10.22037/iej.v19i3.44394] [PMID: 39086712]
4. American Association of Endodontists. Glossary of Endodontic Terms [Internet]. Chicago: AAE; 2020. Available from: https://www.aae.org/specialty/clinical-resources/glossary-endodontic-terms/
5. Pigg M, Brodén J, Fransson H, EndoReCo; the Foresight Research Consortium; Vareman N. How do we and how should we deal with uncertainty in endodontics? Int Endod J. 2022;55(4):282–9. [DOI: 10.1111/iej.13679] [PMID: 34967026]
6. Jakovljevic A, Nikolic N, Jacimovic J, Pavlovic O, Milicic B, Beljic-Ivanovic K, et al. Prevalence of apical periodontitis and conventional nonsurgical root-canal treatment in general adult population: an updated systematic review and meta-analysis of cross-sectional studies published 2012–2020. J Endod. 2020;46(10):1371–86.e8. [DOI: 10.1016/j.joen.2020.07.007] [PMID: 32673634]
7. Duncan HF, Chong BS, Del Fabbro M, El-Karim I, Galler K, Kirkevang LL, et al. Development of European Society of Endodontology S3-level guidelines for the treatment of pulpal and apical disease. Int Endod J. 2021;54(5):643–5. [DOI: 10.1111/iej.13516] [PMID: 33876456]
8. Donnermeyer D, Dammaschke T, Lipski M, Schäfer E. Effectiveness of diagnosing pulpitis: a systematic review. Int Endod J. 2023;56(Suppl 3):296–325. [DOI: 10.1111/iej.13762] [PMID: 35536159]
9. Mainkar A, Kim SG. Diagnostic accuracy of five dental-pulp tests: a systematic review and meta-analysis. J Endod. 2018;44(5):694–702. [DOI: 10.1016/j.joen.2018.01.021] [PMID: 29571914]
10. Gopikrishna V, Tinagupta K, Kandaswamy D. Comparison of electrical, thermal and pulse-oximetry methods for assessing pulp vitality in recently traumatized teeth. J Endod. 2007;33(5):531–5. [DOI: 10.1016/j.joen.2007.01.014] [PMID: 17437866]
11. Kasper RH, Coelho MR, Miguens-Jr SAQ, Grazziotin-Soares R, Barletta FB. Pulse oximetry as a dental-pulp test: a scoping review to identify barriers hindering the use of oximeters in clinical practice. Saudi Dent J. 2024;36(2):262–9. [DOI: 10.1016/j.sdentj.2023.11.006] [PMID: 38419999]
12. Gopikrishna V, Tinagupta K, Kandaswamy D. Evaluation of efficacy of a new custom-made pulse-oximeter dental probe versus electrical and thermal tests for assessing pulp vitality. J Endod. 2007;33(4):411–4. [DOI: 10.1016/j.joen.2006.12.003] [PMID: 17368329]
13. Karayilmaz H, Kirzioğlu Z. Reliability of laser Doppler flowmetry, pulse oximetry and electric-pulp testing in assessing pulp vitality of human teeth. J Oral Rehabil. 2011;38(5):340–7. [DOI: 10.1111/j.1365-2842.2010.02160.x] [PMID: 20868433]
14. Ingólfsson AR, Tronstad L, Hersh EV, Riva CE. Efficacy of laser Doppler flowmetry in determining pulp vitality of human teeth. Endod Dent Traumatol. 1994;10(2):83–7. [DOI: 10.1111/j.1600-9657.1994.tb00065.x] [PMID: 8062812]

15. Adam M. 'Cold is gold'? Diagnostic accuracy of sensibility and vitality testing techniques. Evid Based Dent. 2022;23(4):137. [DOI: 10.1038/s41432-022-0847-5] [PMID: 36526833]
16. Villa-Chávez CE, Patiño-Marín N, Loyola-Rodríguez JP, Zavala-Alonso NV, Martínez-Castañón GA, Medina-Solís CE. Predictive values of thermal and electrical dental-pulp tests: a clinical study. J Endod. 2013;39(8):965–9. [DOI: 10.1016/j.joen.2013.04.019] [PMID: 23880259]
17. Goh L, Er J, Pham Y, Abbott PV. Repeatability of electric-pulp sensibility tests. Aust Endod J. 2022;48(1):20–6. [DOI: 10.1111/aej.12552] [PMID: 34333842]
18. Nemeth L, Birk L, Birk L, Cankar K. Laser-Doppler microvascular flow of dental pulp in relation to caries progression. Lasers Med Sci. 2022;37(3):1549–57. [DOI: 10.1007/s10103-021-03402-1] [PMID: 34420126]
19. Janani K, Ajitha P, Sandhya R, Subbaiyan H, Jose J. Efficiency of a new custom-made pulse-oximeter sensor holder in assessment of actual pulp status. J Family Med Prim Care. 2020;9(7):3333–7. [DOI: 10.4103/jfmpc.jfmpc_73_20] [PMID: 33102292]
20. Dastmalchi N, Jafarzadeh H, Moradi S. Custom-made pulse-oximeter probe versus digital electric-pulp tester, cold spray and rubber cup for assessing pulp vitality. J Endod. 2012;38(9):1182–6. [DOI: 10.1016/j.joen.2012.06.012] [PMID: 22892732]
21. Bender IB, Landau MA, Fonsecca S, Trowbridge HO. Optimum electrode-placement site in electric-pulp testing of 12 anterior teeth. J Am Dent Assoc. 1989;118(3):305–10. [DOI: 10.14219/jada.archive.1989.0096] [PMID: 2921428]
22. Calil E, Caldeira CL, Gavini G, Lemos EM. Determination of pulp vitality in vivo with pulse oximetry. Int Endod J. 2008;41(9):741–6. [DOI: 10.1111/j.1365-2591.2008.01421.x] [PMID: 18554185]
23. Alghaithy RA, Qualtrough AJ. Pulp sensibility and vitality tests for diagnosing pulpal health in permanent teeth: a critical review. Int Endod J. 2017;50(2):135–42. [DOI: 10.1111/iej.12611] [PMID: 26789282]
24. Path MG, Meyer MW. Heterogeneity of blood flow in canine tooth. Arch Oral Biol. 1980;25(2):83–6. [DOI: 10.1016/0003-9969(80)90081-3] [PMID: 6931564]
25. Sasano T, Onodera D, Hashimoto K, Iikubo M, Satoh-Kuriwada S, Shoji N, et al. Possible application of transmitted laser light for assessment of human-pulp vitality. Part 2. Increased laser power for enhanced detection of pulpal blood flow. Dent Traumatol. 2005;21(1):37–41. [DOI: 10.1111/j.1600-9657.2004.00280.x] [PMID: 15660755]
26. Kaur B, Kobayashi Y, Cugini C, Shimizu E. Potential biomarkers for non-invasive diagnosis of pulpal inflammation: a mini review. Front Dent Med. 2021;2:718445. [DOI: 10.3389/fdmed.2021.718445] [PMID: 38947881]
27. Zehnder M, Belibasakis GN. Critical analysis of research methods to study clinical molecular biomarkers in endodontic research. Int Endod J. 2022;55(Suppl 1):37–45. [DOI: 10.1111/iej.13647] [PMID: 34655496]
28. Karrar RN, Cushley S, Duncan HF, Lundy FT, Abushouk SA, Clarke M, El-Karim IA. Molecular biomarkers for objective assessment of symptomatic pulpitis: a systematic review and meta-analysis. Int Endod J. 2023;56(10):1160–77. [DOI: 10.1111/iej.13950] [PMID: 37392154]

Компаративна процена клиничких дијагностичких тестова за испитивање статуса пулпног ткива и утврђивање референтних вредности код здравих младих особа

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САЖЕТАК

Увод/Циљ Традиционалне дијагностичке методе за процену сензибилитета пулпе уобичајено се користе, али им често недостаје прецизност и поузданост, док савремена достигнућа пружају неинвазивне алтернативе, фокусирајући се на проток крви и засићеност пулпе кисеоником. Ова студија је осмишљена са циљем да процени и упореди поузданост свих доступних клиничких метода за процену статуса пулпе и да успостави референтне вредности код здравих младих особа.

Методе Студија пресека обухватила је 25 добровољаца (27,8 ± 5,2 године), односно 150 горњих предњих зуба. Учесници су били здрави, непушачи, са завршеним растом корена, са минималним рестауративним захватима или без њих, пародонтолошки здрави и са радиографски видљивим пулпним коморама. Примењени су различити дијагностички тестови: електро-тест (ЕТ), тест на хладно (ТХ) и тест на топло (ТТ), ласерска доплер-метрија протока крви (ЛДМПК) и пулсна оксиметрија (ПО). Утврђене су референтне вредности.

Резултати Статистички значајне разлике уочене су између свих група зуба (централни секутић, латерални секутић и очњак) за ЕТ, ЛДМПК и ПО. Насупрот томе, ТХ и ТТ нису показали статистички значајне разлике између група зуба. Просечне вредности за све испитанике биле су: ЕТ – 17,7 ± 5,4, ПО – 80,6 ± 1,8% и ЛДМПК – 4,6 ± 1,6 јединица перфузије. Корелациона анализа није показала статистички значајне везе између тестова.

Закључак Савремени дијагностички тестови показују велики потенцијал у пружању поузданијих резултата. Успостављање референтних вредности од суштинског је значаја за унапређење дијагностичке прецизности. Ове вредности не само да ће унапредити клиничко доношење одлука већ ће послужити и као основа за будућа истраживања.

Кључне речи: клиничке дијагностичке методе; ласерска доплер-метрија протока крви; пулсна оксиметрија; електро-тест; зубна пулпа

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Clinical efficacy of different irrigation protocols in teeth with chronic apical periodontitis: randomized clinical trial – a pilot study

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SUMMARY

Introduction/Objective Effective disinfection of the root canal system is essential for successful endodontic treatment. This study aimed to determine the most effective final irrigation protocol.

Methods This randomized clinical trial investigated the antimicrobial efficacy of three irrigation protocols in 30 patients with asymptomatic apical periodontitis in single-rooted teeth. The groups were: (1) 2.5% sodium hypochlorite (NaOCl) + 17% ethylenediaminetetraacetic acid (EDTA); (2) NaOCl + EDTA + 2% chlorhexidine (CHX); and (3) the same as Group 2 with sonic agitation (EndoActivator). Microbial samples were collected at three intervals and analyzed using endpoint and real-time PCR targeting seven key endodontic pathogens.

Results Group 3 demonstrated the greatest bacterial reduction across all time points, with CHX-containing protocols significantly outperforming NaOCl + EDTA alone. Sonic activation further enhanced irrigant efficacy, notably reducing *P. gingivalis*. Despite improvements, complete bacterial eradication was not achieved, and *E. faecalis* and *F. nucleatum* remained persistent.

Conclusion While limitations in microbial sampling and profiling exist, the results underscore the importance of combining chemical agents and mechanical activation to manage complex root canal infections effectively.

Keywords: endodontics; irrigation protocols; PCR; clinical trial

INTRODUCTION

Achieving effective infection control is the primary objective of root canal treatment, as bacteria play a critical role in the development of pulpal and periapical diseases [1]. Due to the complex anatomy of root canals, complete bacterial elimination is nearly impossible [2]. Therefore, endodontic therapy aims to reduce the microbial load to levels that allow periapical healing [1]. This necessitates thorough chemomechanical preparation, combining instrumentation with copious irrigation to remove infected tissue and debris [2].

An ideal irrigant would lubricate instruments, dissolve pulp tissue, eliminate the smear layer, and penetrate complex canal areas – while being antimicrobial, non-toxic, and preserving tooth structure [3]. Since no single irrigant meets all the necessary criteria, it is common to use combinations of irrigants to enhance effectiveness. Sodium hypochlorite (NaOCl) is the most common irrigant due to its strong antimicrobial and tissue-dissolving properties; however, it is highly toxic if extruded beyond the apex [4]. Chlorhexidine digluconate (CHX), while unable to dissolve organic tissue, offers broad-spectrum antibacterial activity, lubrication, and lower cytotoxicity [5]. Ethylenediaminetetraacetic acid (EDTA) aids in smear layer and inorganic debris removal but has limited antibacterial properties [5]. When used together, these irrigants can effectively address each other's limitations.

Irrigation techniques include syringe irrigation, ultrasonic activation, and sonic agitation [1]. Activation methods enhance the effectiveness of irrigants, particularly in complex anatomies, by improving smear layer removal and bacterial reduction [1]. Newer systems employing plastic rotary instruments apply similar principles [6].

Given the ongoing issues in the field of Endodontology, this study aimed to determine

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the most effective final irrigation protocol for reducing bacterial presence in root canals after chemo-mechanical preparation.

METHODS

Patient selection and eligibility criteria

A single-blinded, two-arm randomized clinical trial, in the form of a pilot investigation, evaluated the effectiveness of three final irrigation protocols in eliminating root canal bacteria after chemo-mechanical preparation. One clinician with three years of endodontic experience treated 30 patients (mean age 51.2), with asymptomatic apical periodontitis at the Department of Dental Medicine, Faculty of Medicine, University of Novi Sad (April–December 2022). Patients had single-rooted teeth with necrotic pulp and carious lesions. Exclusion criteria included prior endodontic treatment, periodontal pockets > 4 mm, active orthodontic treatment, fractures, or recent antibiotic use. Data analysis was blinded. All patients were thoroughly informed about the procedures involved, and written consent was obtained along with an assent document from every participant in this study.

Sample collection and root canal treatment procedures

Samples were collected under strict aseptic conditions. After scaling and cleaning with pumice, a rubber dam was applied, and caries or prior restorations were removed under saline irrigation. The tooth was disinfected with 3% hydrogen peroxide, followed by 2.5% sodium hypochlorite (NaOCl) (i-dental, Šiauliai, Lithuania), and then neutralized with 10% sodium thiosulfate ($Na_2S_2O_3$) (Centrochem, Stara Pazova, Serbia). These steps were repeated during access cavity preparation to prevent contamination. A sterile paper point (Dentsply Maillefer, Ballaigues, Switzerland) was carefully inserted without contacting canal walls [7], left for 60 seconds, and stored in a sterile 1.5 ml tube labeled S1, then frozen at -20°C.

Working length was determined using an apex locator (Propex, Dentsply Maillefer, Ballaigues, Switzerland) and radiographs. The canal was shaped with endodontic rotary instruments (ProTaper Next, Dentsply Maillefer, Ballaigues, Switzerland) and irrigated with 9 ml of 2.5% NaOCl. Upon reaching the working length, one of three randomized irrigation protocols was applied. A second sterile paper point (S2) was inserted 1 mm short of working length for 60 seconds, then stored as described.

A sterile cotton pellet and temporary filling were placed (Orafil-G, Prevest DenPro, Bari Brahmana, Jammu, Jammu and Kashmir, India). After 48 hours, the tooth was disinfected again, and the temporary restoration removed. Sampling was repeated (S3). The assigned irrigation protocol was reapplied, the canal dried, and calcium hydroxide medication placed (i-CAL, i-dental, Šiauliai, Lithuania).

Ten days later, if asymptomatic, the medication was removed and the canal obturated with gutta-percha and sealer. If symptoms persisted, the procedure was repeated. A permanent filling was placed 24 hours post-obturation [8].

Irrigation protocols

All irrigation protocols were performed using 5 mL Luer-lock syringes and closed-ended endodontic needles (i-TIPS, i-dental, Šiauliai, Lithuania), and included sterile saline irrigation between irrigants, to prevent mixing of different irrigants and subsequent chemical reactions between them.

Group 1 (NaOCl, EDTA): During treatment, the root canal was rinsed with 9 ml total amount of 2.5% NaOCl. Upon treatment completion, the canal was rinsed with 6 mL of 2.5% NaOCl and 3 ml of 17% EDTA (Cerkamed, Stalowa Wola, Poland), alternately applying 2 mL of NaOCl and 1 mL of EDTA, until the entire amount of both irrigants was consumed. Next, 1 mL of 5% $Na_2S_2O_3$ (Centrochem, Stara Pazova, Serbia) was added to the canal to inactivate the remaining NaOCl.

Group 2 (NaOCl, EDTA, CHX): The previously explained irrigation protocol (Group 1) is enriched with the application of a 2% chlorhexidine (CHX) solution. The irrigation commenced with a combination of 6 ml of 2% CHX (Cerkamed, Stalowa Wola, Poland) and 3 ml of 17% EDTA, alternately applying 2 ml of CHX and 1 ml of EDTA until both irrigants were consumed. Finally, the canal was flushed with 1 ml of 10% $Na_2S_2O_3$ to inactivate the remaining amount of CHX.

Group 3 *(*NaOCl, EDTA, CHX + endoactivation*)*: Same as Group 2, but with sonic activation using the Endoactivator® (Dentsply Sirona, Charlotte, NC, USA) (30 seconds per irrigant). Activation enhanced irrigant efficacy. Tip size matched prepared canal diameter, one size smaller, operating at 2–3 kHz [9].

Polymerase chain reaction (PCR) analyses

The collected intracanal fluid samples (S1–S3) were used for qualitative and quantitative analysis of the bacterial communities present in the root canal system. Bacterial DNA was extracted by heating the samples in 300 µl of 50 mM NaOH solution in a thermoblock (Biosan TS100C, Riga, Latvia) at 95°C for 5 minutes. After heating, 30 µl of a 1 M tris-(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl) solution (pH 8) was added to the samples to stabilize and preserve the isolated DNA molecules. Isolated DNA samples were stored at -20°C until further analyses [10].

A qualitative analysis of the bacterial composition of the root canal system was performed using the end-point PCR method with specific primers, allowing parts of the DNA molecule that are specific for each tested bacterium to be multiplied (Figure 1). The presence of the following bacteria was assessed: *Aggregatibacter*

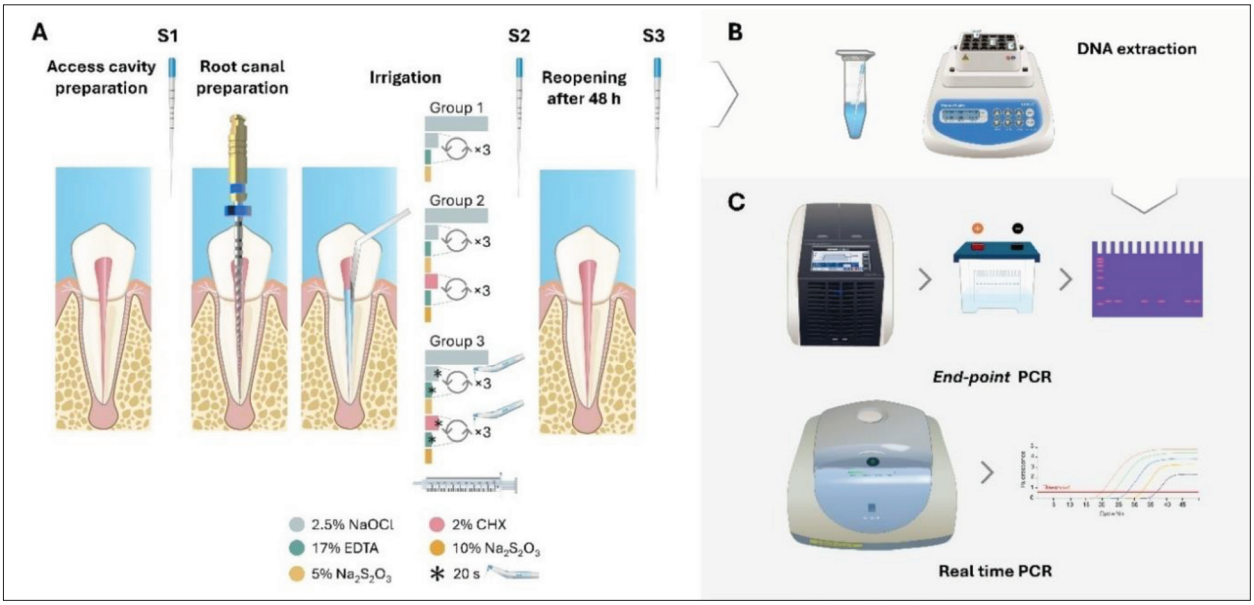

Figure 1. Experimental flow chart: A) sampling; B) DNA extraction; C) DNA amplification and visualization

Table 1. Primers used for PCR and their product sizes

Species	Sequence (5′-3′)	Product size (bp)
Aggregatibacter actinomycetemcomitans	Fwd GCTAATACCGCGTAGAGTCGG Rv ATTTCACACCTCACTTAAAGGT	500
Porphyromonas gingivalis	Fwd AGGCAGCTTGCCATACTGCG Rv ACTGTTAGCAACTACCGATGT	404
Porphyromonas endodontalis	Fwd GCTGCAGCTCAACTGTAGTC Rv CCGCTTCATGTCACCATGTC	665
Prevotella intermedia	Fwd CGTGGACCAAAGATTCATCGGTGGA Rv CCGCTTTACTCCCCAACAAA	259
Fusobacterium nucleatum	Fwd ATGGTGGCTAAAAATTATAGT Rv ACCCTCACTTTGAGGATTATA	1000
Parvimonas micra	Fwd AGAGTTTGATCCTGGGCTCAG Rv ATATCATGCGATTCTGTGGTCTC	207
Enterococcus faecalis	Fwd TACTGACAAACCATTCATGATG Rv AACTTCGTCACCAACGCGAAC	112

actinomycetemcomitans (Aa), *Porphyromonas gingivalis* (Pg), *Porphyromonas endodontalis* (Pe), *Prevotella intermedia* (Pi), *Fusobacterium nucleatum* (Fn), *Parvimonas micra* (Pm), and *Enterococcus faecalis* (Ef). Five microliters of bacterial DNA were resuspended in 20 µl of aqueous mixture containing 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U of DNA polymerase (all products from Thermo Fisher Scientific™, Waltham, MA, USA) and 0.2 µM of specific primers and used for DNA amplification in a thermal cycler (PeqSTAR 2X®, Peqlab, Erlangen, Germany). The PCR started with an initial denaturation step (95°C for 3 minutes) followed by 35 cycles of denaturation (95°C for 45 seconds), annealing (55°C for 1 minute) and elongation (72°C for 1 min), and a final elongation step (72°C for 5 minutes). PCR products were separated on a polyacrylamide gel using a vertical electrophoresis system (PerfectBlue™ Twin S system, Peqlab, Erlangen, Germany) and visualized by staining with a 1% ethidium bromide solution on a UV transilluminator (TCX-15 MX®, Vilber Lourmat, Collégien, France). The list of primer sequences and sizes of PCR products is given in Table 1.

Real-time PCR was used for the quantitative analysis of the bacterial communities present in the root canal system. This method relies on fluorescence detection and requires primers specific for highly conserved regions of the 16S ribosomal DNA of bacteria. The components of the reaction mixture were as follows: two microliters of bacterial sample, 10 µl of PCR master mix (Luna® Universal qPCR Master Mix 2X, New England Biolabs, Ipswich, MA, USA), 1 µl of forward and reverse primer (final concentration in solution for each primer was 250 nM) and ultra-pure nuclease-free water up to 20 µl in total. The sequences of the forward and reverse primers were TCCTACGGGAGCACAGT and GGACTACCAGGGTATCTAATCCTGTT, respectively. A reaction was performed on a fluorescence detection system (Line Gene-K fluorescence PCR detection system, BIOER Technology, Shanghai, China) under the following conditions: initial denaturation (95°C for 5 minutes), 35 cycles of denaturation (95°C for 1 minute), annealing (55°C for 1 minute) and elongation (72°C for 1.5 min), and a final elongation step (72°C for 5 min). A standard curve, generated from seven (1:10) serial dilutions of DNA samples of the reference bacterial strain *Prevotella melaninogenica* ATCC 25845 (Microbiologics KWIK-STIK, Manassas, VA, USA), was used for extrapolation of the total gene copy number (total bacterial count) [11].

Statistical analysis

Categorical data were presented as absolute numbers; numerical data as means, standard deviations, medians, and ranges. The χ^2 test compared bacterial species frequency across protocols at different time points. The

Table 2. Prevalence of bacteria in each group at different time points

Time point	Bacteria	Irrigation Protocol 1: NaOCl + EDTA	Irrigation Protocol 2: NaOCl + EDTA + CHX	Irrigation Protocol 3: NaOCl + EDTA + CHX + activation	p
First	*Aa*	1 (12.5%)	1 (9.1%)	1 (9.1%)	0.963
	Pg	0 (0%)	2 (18.2%)	4 (36.4%)	0.145
	Fn	0 (0%)	5 (45.5%)	4 (36.4%)	0.087
	Pe	3 (37.5%)	2 (18.2%)	3 (27.3%)	0.642
	Ef	4 (50%)	6 (54.5%)	6 (54.5%)	0.976
	Pm	2 (25%)	5 (45.5%)	1 (9.1%)	0.155
	Pi	0 (0%)	0 (0%)	0 (0%)	N/A
Second	*Aa*	0 (0%)	1 (9.1%)	1 (9.1%)	0.677
	Pg	1 (12.5%)	1 (9.1%)	0 (0%)	0.515
	Fn	1 (12.5%)	1 (9.1%)	3 (27.3%)	0.485
	Pe	1 (12.5%)	0 (0%)	1 (9.1%)	0.515
	Ef	6 (75%)	8 (72.7%)	6 (54.5%)	0.560
	Pm	2 (25%)	1 (9.1%)	1 (9.1%)	0.526
	Pi	0 (0%)	0 (0%)	0 (0%)	N/A
Third	*Aa*	2 (25%)	1 (9.1%)	1 (9.1%)	0.526
	Pg	0 (0%)	1 (9.1%)	0 (0%)	0.409
	Fn	3 (37.5%)	5 (45.5%)	5 (45.5%)	0.927
	Pe	1 (12.5%)	0 (0%)	1 (9.1%)	0.515
	Ef	4 (50%)	6 (54.5%)	4 (36.4%)	0.677
	Pm	1 (12.5%)	1 (9.1%)	2 (18.2%)	0.819
	Pi	2 (25%)	0 (0%)	0 (0%)	0.053

EDTA – ethylenediaminetetraacetic acid; CHX – chlorhexidine; *Aa – Aggregatibacter actinomycetemcomitans*; *Pg – Porphyromonas gingivalis*; *Pe – Porphyromonas endodontalis*; *Pi – Prevotella intermedia*; *Fn – Fusobacterium nucleatum*; *Pm – Parvimonas micra*; *Ef – Enterococcus faecalis*

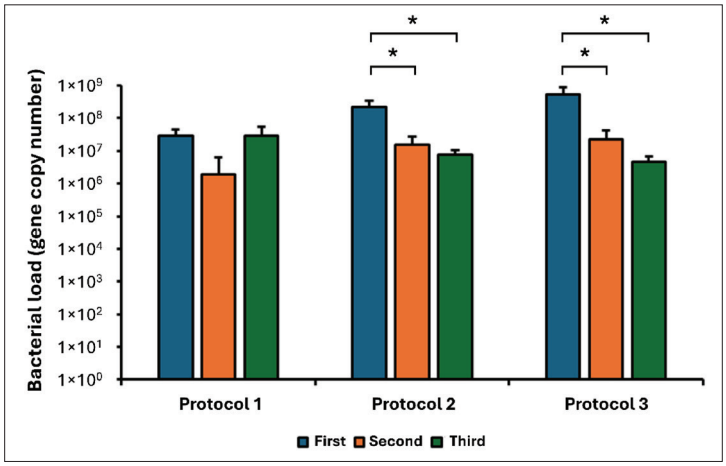

Figure 2. Total bacterial load (gene copy number) in different study groups assessed by real-time PCR;

*statistically significant ($p < 0.05$)

Kruskal–Wallis test assessed total bacterial counts between protocols within each period. The Friedman test analyzed bacterial count changes over time within protocols; if significant, the Wilcoxon test was used for pairwise comparisons. A p-value < 0.05 indicated statistical significance. Data analysis was performed using IBM SPSS Statistics, Version 28.0 (IBM Corp., Armonk, NY, USA).

Ethics: The study received ethical approval from the Faculty of Medicine, University of Novi Sad (01-39/183/1, 2022).

RESULTS

At the initial measurement, no statistically significant differences were found among the three irrigation protocols regarding bacterial prevalence. *Aggregatibacter actinomycetemcomitans* (*Aa*) was detected in one case per group. *Porphyromonas gingivalis* (*Pg*) and *Fusobacterium nucleatum* (*Fn*) were absent in the first protocol (NaOCl + EDTA). In the second protocol (NaOCl + EDTA + CHX), *Pg* appeared in 18.2% and *Fn* in 45.5% of cases. The third protocol (NaOCl + EDTA + CHX + activation) showed both *Pg* and *Fn* in 36.4% of cases. *Prevotella intermedia* (*Pe*) was most prevalent in the first group (37.5%), followed by 18.2% and 27.3% in the second and third groups, respectively. *Enterococcus faecalis* (*Ef*) was the most frequently isolated bacteria: 50% in the first group and 54.5% in both the second and third. *Parvimonas micra* (*Pm*) was most common in the second group (45.5%), with 25% in the first and 9.1% in the third. *Pi* was absent at this time but was detected in 25% of cases in the first group during the third time point.

During the second measurement, no significant intergroup differences were observed. *Aa* was absent in the first group and present in one case in both the second and third. *Pg* appeared in one case each in the first and second groups. *Fn* had its highest prevalence (27.3%) in the third group. *Ef* increased in the first group to 75% and was found in 72.7% of cases in the second group, while remaining unchanged in the third. *Pm* prevalence stayed the same in the first and third groups but dropped significantly in the second (45.5–9.1%). *Pi* was not detected.

In the third measurement, bacterial prevalence again showed no statistically significant differences between groups. *Aa* was found in 25% of cases in the first group but unchanged in the other groups. *Pg* was absent in the first and third groups but stable in the second. *Fn* increased across all protocols compared to the second period, notably 37.5% in the first group. *Pe* levels remained stable between the second and third periods. *Ef* decreased in the third period, returning to levels seen initially, with a notable drop in the activation group (54.5–36.4%). *Pm* prevalence fell in the first group (25–12.5%), remained unchanged in the second, and rose in the third group to 18.2%. *Pi* was again found only in the first group (25%) (Table 2).

Overall, there were no statistically significant differences in total bacterial counts between protocols at any time point. Intra-group analysis showed significant reductions in bacterial counts from the first to second and first to third measurements in the second and third protocols. However, no significant changes occurred between the second and third periods in either group. Despite a rise in bacterial counts after the second measurement, the final levels remained significantly lower than at baseline (Figure 2).

DISCUSSION

Biofilms are resilient bacterial communities embedded in a matrix that protects them from host defenses and environmental stressors. Their ability to persist in root canals poses significant challenges during endodontic treatment. This study evaluated three irrigation protocols for biofilm elimination: (1) NaOCl with EDTA, (2) NaOCl with EDTA and CHX, and (3) NaOCl with EDTA and CHX with sonic agitation. Although no statistically significant differences were found among the protocols regarding bacterial prevalence, the second and third protocols reduced the total bacterial load more effectively. The analysis confirmed the presence of an intracanal microbial community characteristically associated with endodontic environments [12]. However, their prevalence remained largely unchanged under the irrigation protocols applied, confirming the strong resilience due to numerous virulence factors they possess [13].

Despite intervention, key pathogens including *E. faecalis* remained prevalent due to factors such as deep dentinal tubule invasion, biofilm maturity, and dentin demineralization, which can mineralize the matrix and reduce irrigation efficacy [14]. *E. faecalis*, a well-known endodontic pathogen [15], resists NaOCl and CHX in biofilms even though both irrigants are highly effective in suspension. High concentrations of NaOCl (5.25%) can eliminate *E. faecalis* rapidly in planktonic form, while CHX at 2% is comparably effective but requires longer contact in biofilms [16].

Combined NaOCl and CHX solutions demonstrated enhanced antimicrobial activity in suspension, but little is known about their synergistic impact on biofilms. In ex vivo models, *E. faecalis* penetrates dentin deeply, beyond the reach of conventional irrigants [17]. Agitation increases irrigant penetration, but may also drive bacteria further into tubules [18]. EDTA, though helpful in smear layer removal, has minimal antimicrobial action against *E. faecalis* [19].

Clinical studies report inconsistent *E. faecalis* prevalence, ranging from 14% to 92% [20]. Our study found a 53% initial prevalence. Notably, in groups 1 and 2, prevalence increased post-treatment, likely due to bacterial release from tubules during instrumentation [21]. This aligns with other studies showing recolonization even after confirmed eradication [20]. Group 3, with sonic activation, showed an 18% reduction after 48 hours, but differences between protocols were not statistically significant. This result aligns with findings from Brito et al., who reported comparable reductions in *E. faecalis* when comparing conventional irrigation to endo-activated methods [22].

F. nucleatum was the second most common species identified. Known for bridging oral biofilms and resisting environmental stress, its eradication is difficult. Found in 30% of initial samples, its prevalence was lower than in some literature, possibly due to antagonism with *E. faecalis* [23]. Although NaOCl and CHX reduce *F. nucleatum* in biofilms, complete eradication remains elusive, potentially due to buffering by tissue fluids or irrigant inactivation upon dentin contact. Its resurgence post-treatment may reflect recolonization [24].

P. endodontalis, another Gram-negative anaerobe, was present in 26.6% of cases – comparable to prior studies [25]. Post-treatment, its prevalence dropped significantly or was undetectable, with no further change after 48 hours, indicating good responsiveness to the protocols used.

P. gingivalis, a major oral and systemic pathogen, showed 20% prevalence. In protocol 3 (with sonic activation), it was completely undetectable after treatment, suggesting sonic agitation may help eliminate this species. In groups 1 and 2, prevalence decreased but persisted.

P. intermedia was undetectable initially and post-treatment across all protocols but emerged in group 1 after 48 hours. This reappearance may indicate recolonization due to instrumentation displacing bacteria [23, 24].

The grouping of *P. endodontalis*, *P. gingivalis*, and *P. intermedia* is common due to similar antimicrobial sensitivity. In previous in vivo studies, both NaOCl and CHX significantly reduced these species, though CHX was slightly less effective [26]. Differences with our findings may result from varying concentrations and exposure times.

P. micra, an anaerobic Gram-positive bacterium, appeared in 23% of patients. Its reduction was most notable in group 2, suggesting greater CHX sensitivity. Literature supports this, indicating CHX produces a larger inhibition zone for *P. micra* than NaOCl [27].

A. actinomycetemcomitans was rare (10%) and inconsistently affected by irrigation. It reappeared post-treatment in group 1 but remained stable in groups 2 and 3. This pathogen is often found in periodontal infections and has limited interaction with other biofilm species.

Regarding overall bacterial load, protocols 2 and 3 showed significant reductions immediately post-treatment, with further reductions at the 48-hour mark. In contrast, protocol 1 showed possible recolonization. This suggests CHX's prolonged antimicrobial activity and the potential benefit of agitation [28].

While traditional syringe irrigation with NaOCl or CHX effectively reduces bacterial load, several studies, including ours, suggest CHX may offer superior bacterial reduction due to its substantivity and prolonged effect. Some ex vivo studies also indicate that sonic agitation enhances NaOCl effectiveness, particularly in lateral canal areas where bacteria persist [29]. However, results remain mixed, and other studies found no difference between conventional and sonic irrigation [30]. Our findings suggest agitation does not consistently outperform manual irrigation in clinical settings. Nonetheless, enhanced performance may be achieved by optimizing irrigant chemistry, oscillation frequency, and duration, particularly in complex canal systems.

Several study limitations should be noted. Firstly, sampling precision *in vivo* is constrained by root canal anatomy and the use of paper points, which may not reflect the true microbial composition. Additionally, bacteria residing deep in dentin cannot be accessed without more advanced, often impractical techniques. Secondly, sonic activation loses effectiveness when files contact the canal

walls. Thirdly, real-time PCR, while sensitive, only detects specific bacteria and total counts, limiting full microbiome characterization. Finally, ethical constraints precluded the use of negative controls like saline, which are standard in controlled experimental designs.

CONCLUSION

Traditional irrigants like NaOCl and CHX, whether used alone or combined, reduce bacterial prevalence but fail to achieve complete eradication. *E. faecalis* and *F. nucleatum* remain particularly resistant due to their ability to persist in biofilms and dentin. CHX appears more effective than NaOCl due to its longer-lasting effects. Sonic activation modestly improves results, though its benefits are not always statistically significant. Future strategies should focus on optimizing irrigant properties, improving agitation methods, and employing comprehensive molecular techniques to fully characterize and address endodontic biofilms.

Conflict of interest: None declared.

REFERENCES

1. Boutsioukis C, Arias-Moliz MT. Present status and future directions – irrigants and irrigation methods. Int Endod J. 2022;55(Suppl 3):588–612. [DOI: 10.1111/iej.13739] [PMID: 35338652]
2. Prada I, Mico-Munoz P, Giner-Lluesma T, Mico-Martinez P, Muwaquet-Rodriguez S, Albero-Monteagudo A. Update of the therapeutic planning of irrigation and intracanal medication in root canal treatment: a literature review. J Clin Exp Dent. 2019;11(2):e185–93. [DOI: 10.4317/jced.55560] [PMID: 30805124]
3. Park KH, Ordinola-Zapata R, Noblett WC, Lima BP, Staley C. The effect of ultrasonic and multisonic irrigation on root canal microbial communities: an ex vivo study. Int Endod J. 2024;57(7):895–906. [DOI: 10.1111/iej.13996] [PMID: 37983635]
4. Baruwa AO, Martins JNR, Maravic T, Mazzitelli C, Mazzoni A, Ginjeira A. Effect of endodontic irrigating solutions on radicular dentine structure and matrix metalloproteinases – a comprehensive review. Dent J (Basel). 2022;10(12):219. [DOI: 10.3390/dj10120219] [PMID: 36547035]
5. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389–98. [DOI: 10.1016/j.joen.2005.09.014] [PMID: 16631834]
6. Chalub LO, Nunes GP, Strazzi-Sahyon HB, Ferrisse TM, Dos Santos PH, Gomes-Filho JE, et al. Antimicrobial effectiveness of ultrasonic irrigation in root canal treatment: a systematic review of randomized clinical trials and meta-analysis. Clin Oral Investig. 2023;27(4):1343–61. [DOI: 10.1007/s00784-023-04897-4] [PMID: 36757461]
7. Rôças IN, Provenzano JC, Neves MAS, Siqueira JF. Disinfecting effects of rotary instrumentation with either 2.5% sodium hypochlorite or 2% chlorhexidine as the main irrigant: a randomized clinical study. J Endod. 2016;42(6):943–7. [DOI: 10.1016/j.joen.2016.03.019] [PMID: 27142579]
8. Rôças IN, Provenzano JC, Neves MS, Alves FRF, Gonçalves LS, Siqueira JF Jr. Effects of calcium hydroxide paste in different vehicles on bacterial reduction during treatment of teeth with apical periodontitis. J Endod. 2023;49(1):55–61. [DOI: 10.1016/j.joen.2022.10.008] [PMID: 36309246]
9. Hoedke D, Kaulika N, Dommisch H, Schlafer S, Shemesh H, Bitter K. Reduction of dual-species biofilm after sonic- or ultrasonic-activated irrigation protocols: a laboratory study. Int Endod J. 2021;54(12):2219–28. [DOI: 10.1111/iej.13618] [PMID: 34418114]
10. Saab YB, Kabbara W, Chbib C, Gard PR. Buccal cell DNA extraction: yield, purity, and cost – a comparison of two methods. Genet Test. 2007;11(4):413–6. [DOI: 10.1089/gte.2007.0044] [PMID: 18294058]
11. Brajović G, Popović B, Puletić M, Kostić M, Milašin J. Estimation of total bacteria by real-time PCR in patients with periodontal disease. Srp Arh Celok Lek. 2016;144(1–2):10–4. [DOI: 10.2298/sarh1602010b] [PMID: 27276852]
12. Siqueira JF, Rôças IN. Present status and future directions: microbiology of endodontic infections. Int Endod J. 2022;55(Suppl 3):512–30. [DOI: 10.1111/iej.13677] [PMID: 34958494]
13. Sharma G, Garg N, Hasan S, Shirodkar S. Prevotella: an insight into its characteristics and associated virulence factors. Microb Pathog. 2022;169:105673. [DOI: 10.1016/j.micpath.2022.105673] [PMID: 35843443]
14. Ran S, Wang J, Jiang W, Zhu C, Liang J. Assessment of dentinal tubule invasion capacity of Enterococcus faecalis under stress conditions ex vivo. Int Endod J. 2015;48(4):362–72. [DOI: 10.1111/iej.12322] [PMID: 24872016]
15. Alghamdi F, Shakir M. The influence of Enterococcus faecalis as a dental root canal pathogen on endodontic treatment – a systematic review. Cureus. 2020;12(3):e7257. [DOI: 10.7759/cureus.7257] [PMID: 32292671]
16. Ruksakiet K, Hanák L, Farkas N, Hegyi P, Sadaeng W, Czumbel LM, et al. Antimicrobial efficacy of chlorhexidine and sodium hypochlorite in root canal disinfection: a systematic review and meta-analysis of randomized controlled trials. J Endod. 2020;46(8):1032–41.e7. [DOI: 10.1016/j.joen.2020.05.002] [PMID: 32413440]
17. Hahn CL, Hanford K. An in vitro model to study the colonization and tubular invasion of Enterococcus faecalis. J Endod. 2021;47(3):451–7. [DOI: 10.1016/j.joen.2020.12.004] [PMID: 33359252]
18. Prasad AB, Raisingani D, Srivastava H, Dadhich S, Vijaywargiya S, Moryani V. Comparative evaluation of penetration depth of irrigants into root dentin after manual sonic and ultrasonic activation using dye penetration method under light microscope – an in vitro study. Int J Clin Pediatr Dent. 2024;16(Suppl 3):S253–7. [DOI: 10.5005/jp-journals-10005-2688] [PMID: 38268638]
19. Swimberghe RCD, Crabbé A, De Moor RJG, Coenye T, Meire MA. Model system parameters influence the sodium hypochlorite susceptibility of endodontic biofilms. Int Endod J. 2021;54(9):1557–70. [DOI: 10.1111/iej.13544] [PMID: 33932297]
20. Vianna ME, Horz HP, Conrads G, Feres M, Gomes BPFA. Comparative analysis of endodontic pathogens using checkerboard hybridization in relation to culture. Oral Microbiol Immunol. 2008;23(4):282–90. [DOI: 10.1111/j.1399-302X.2007.00425.x] [PMID: 18582327]
21. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med. 2002;13(2):171–83. [DOI: 10.1177/154411130201300207] [PMID: 12097359]
22. Zeng C, Hu P, Egan CP, Bergeron BE, Tay F, Ma J. Bacteria debridement efficacy of two sonic root canal irrigant activation systems. J Dent. 2024;140:104770. [DOI: 10.1016/j.jdent.2023.104770] [PMID: 37923053]
23. Xiang D, Dong PT, Cen L, Bor B, Lux R, Shi W, et al. Antagonistic interaction between two key endodontic pathogens Enterococcus faecalis and Fusobacterium nucleatum. J Oral Microbiol. 2023;15(1):2149448. [DOI: 10.1080/20002297.2022.2149448] [PMID: 36452179]
24. Nogales CG, Cazares RXR, Nardello LCL, Mayer MPA, Gavini G, Zehnder M, et al. Evaluating the impact of ultrasonic irrigation on bacterial levels and activity following chemomechanical procedures. J Endod. 2025;51(2):118–23. [DOI: 10.1016/j.joen.2024.09.001] [PMID: 39276864]
25. Gomes BPFA, Jacinto RC, Pinheiro ET, Sousa ELR, Zaia AA, Ferraz CCR, et al. Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia and Prevotella nigrescens in endodontic lesions detected by culture and by PCR. Oral Microbiol Immunol. 2005;20(4):211–5. [DOI: 10.1111/j.1399-302X.2005.00214.x] [PMID: 15943764]
26. Alquria TA, Acharya A, Tordik P, Griffin I, Martinho FC. Impact of root canal disinfection on the bacteriome present in primary endodontic infection: a next-generation sequencing study. Int Endod J. 2024;57(8):1124–35. [DOI: 10.1111/iej.14074] [PMID: 38700876]

27. Briseño-Marroquín B, Callaway A, Shalamzari NG, Wolf TG. Antibacterial efficacy of peracetic acid in comparison with sodium hypochlorite or chlorhexidine against Enterococcus faecalis and Parvimonas micra. BMC Oral Health. 2022;22(1):119. [DOI: 10.1186/s12903-022-02148-8] [PMID: 35397605]
28. Fedorowicz Z, Nasser M, Sequeira-Byron P, de Souza RF, Carter B, Heft M. Irrigants for non-surgical root canal treatment in mature permanent teeth. Cochrane Database Syst Rev. 2012;(9):CD008948. [DOI: 10.1002/14651858.CD008948.pub2] [PMID: 22972129]
29. Ada KS, Shetty S, Jayalakshmi K, Nadig P, Manje Gowda P, Selvan A. Influence of different irrigant activation methods on apical debris extrusion and bacterial elimination from infected root canals. J Conserv Dent. 2023;26(1):31. [DOI: 10.4103/jcd.jcd_378_22] [PMID: 36908725]
30. Kishen A, Shrestha A, Del Carpio-Perochena A. Validation of biofilm assays to assess antibiofilm efficacy in instrumented root canals after syringe irrigation and sonic agitation. J Endod. 2018;44(2):292–8. [DOI: 10.1016/j.joen.2017.10.005] [PMID: 29254815]

Клиничка ефикасност различитих иригационих протокола код зуба са хроничним апикалним периодонтитисом: рандомизовано клиничко испитивање – пилот-студија

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САЖЕТАК

Увод/Циљ Ефикасна дезинфекција система канала корена кључна је за успешан ендодонтски третман. Циљ ове студије био је да се утврди најефикаснији коначни протокол испирања канала корена зуба.

Методе У овом рандомизованом клиничком испитивању испитивана је антимикробна ефикасност три различита иригациона протокола код 30 пацијената са асимптоматским апикалним периодонтитисом једнокорених зуба. Групе су биле следеће: (1) 2,5% натријум-хипохлорит (*NaOCl*) + 17% *EDTA*; (2) *NaOCl* + *EDTA* + 2% хлорхексидин (*CHX*); и (3) исто као група 2, уз додатак соничне активације (*EndoActivator*). Микробни узорци су прикупљени у три фазе и анализирани помоћу *PCR* методе (класичне и *real-time*) са циљем детекције седам кључних ендодонтских патогена.

Резултати Група 3 је показала највеће смањење бактеријског присуства у свим фазама, док су протоколи са *CHX* били значајно ефикаснији од комбинације *NaOCl* + *EDTA*. Сонична активација додатно је побољшала деловање ириганаса, посебно против *P. gingivalis*. Ипак, потпуна ерадикација бактерија није постигнута, а *E. faecalis* и *F. nucleatum* су остали перзистентни.

Закључак Резултати указују на значај комбинације хемијских агенаса и механичке активације у дезинфекцији комплексних система канала.

Кључне речи: ендодонција; протоколи иригације; *PCR*; клиничка студија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Expression of terminal galactose and sialic acid on serum IgA in IgA multiple myeloma

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SUMMARY

Introduction/Objective IgA multiple myeloma has a poor prognosis, and altered glycosylation of myeloma IgA may be one of contributing factors. This study examined the expression of terminal galactose and sialic acid (SA) on serum IgA oligosaccharides in patients with IgA myeloma, compared to healthy control sera.

Methods Serum samples from 15 IgA myeloma patients and pooled serum from 100 healthy donors were analyzed. IgA was purified using peptide M affinity chromatography. Terminal galactose and SA expression on isolated IgA was analyzed by *Ricinus communis* agglutinin I and *Sambucus nigra* agglutinin lectin blotting.

Results IgA-heavy chains from both healthy individuals and all myeloma patients expressed galactose. SA was present in healthy control and in 14 out of 15 myeloma patients. Compared to controls, myeloma IgA showed 12–63% a reduction in galactose and a 67–97% reduction in SA expression on heavy chains. Notable galactosylation of IgA-light chains was observed in only three, while weak SA expression was seen in 14 myeloma cases. Healthy IgA was predominantly monomeric and expressed both galactose and SA. Myeloma IgA existed in both and polymeric forms expressing detectable galactose level, though with different expression levels among individuals. At the same time, SA was undetectable.

Conclusion The results of this study showed altered glycosylation of myeloma IgA. Compared to healthy control, myeloma IgA-heavy chains expressed reduced terminal galactose and SA. Notable galactosylation of light chains was observed in three cases. Unlike SA, galactose was detectable on intact monomeric and polymeric multiple myeloma IgA.

Keywords: multiple myeloma; IgA; glycosylation; sialic acid; galactose

INTRODUCTION

Multiple myeloma is an immunoproliferative disease characterized by the accumulation of malignant plasma cells in the bone marrow. The presence and the level of monoclonal immunoglobulins, known as M-proteins or paraproteins, in serum and urine are related to the outcome of patients with myeloma. IgA paraprotein occurs in up to 20% of myeloma cases [1]. Although IgA myeloma is rarer than IgG, the clinical course is more aggressive with a prognostically less favorable outcome compared to IgG isotype [1].

Immunoglobulins are glycoproteins, and the presence of oligosaccharides of fragment crystallizable region (Fc region) is essential in regulating the immune response [2]. IgA is one of the most heavily glycosylated antibodies. Unlike IgG, which possesses a single conserved N-glycosylation site, IgA is characterized by multiple N-glycosylation sites. Additionally, IgA1 possesses nine potential O-glycosylation sites located in its hinge region. To date, 16 N-linked glycan structures on IgA, exhibiting significant heterogeneity due to variations in terminal galactose and sialic acid content, have been identified [3, 4].

The IgA glycans have been shown to have a significant effect on its immune function [5]. Paraproteins may have an altered carbohydrate profile compared to normal serum immunoglobulins of the same isotype. Renfrow et al. [5, 6] showed that the decrease in galactose content of IgA1 subclass myeloma is the result of aberrant galactosylation of O-linked glycans in the hinge region. Additionally, Bosseboeuf et al. [7] showed that sialic acid expression in myeloma IgA was reduced compared to healthy individuals. However, the location of sialylated oligosaccharides on IgA molecules was not analyzed in this study. Some of the complications in IgA myeloma may be linked to the structural characteristics of monoclonal IgA, including the expression of glycans on their α-heavy chains of both monomeric and polymeric forms of IgA. Given the importance of pathogenic potential of immunoglobulin glycans, the primary aim of the study was to assess the expression of terminal galactose and sialic acid on heavy and light chains of IgA molecules isolated from sera of multiple myeloma patients

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and to compare it with the expression of these monosaccharides on IgA from healthy individuals.

METHODS

Serum samples

Pooled serum samples of 100 healthy blood donors from the Institute of Transfusion and Haemobiology, Military Medical Academy were used in this study. Ethical approval (No. 17/2020) was granted by the Military Medical Academy Ethics Committee. Serum from 15 individuals with multiple myeloma (C90.0 – *myeloma multiplex*) was used with ethical approval (No. 213/23) from the Institute for Blood Transfusion of Serbia Ethics Committee.

Paraprotein detection and the isotype analysis

Serum samples from multiple myeloma patients were analyzed during diagnostics at the Institute for Blood Transfusion of Serbia. The presence of paraproteins was confirmed by serum protein agarose gel electrophoresis and immunochemically identified by immunoelectrophoresis [8, 9]. The concentrations of IgG, IgA, and IgM in myeloma sera were determined by immunonephelometry on the Siemens BN ProSpec System (Siemens Healthineers, Erlangen, Germany) analyzer. The subclass of IgA paraproteins was determined by dot blot at the Institute for Medical Research [10].

Isolation of IgA

Myeloma patients and healthy donors' serum IgA was isolated by affinity chromatography on peptide M agarose (InvivoGen, San Diego, CA, USA) [11]. Chromatography was performed according to the manufacturer's instruction. Protein concentration was determined with the NanoPhotometer P-330 (Implen GmbH, München, Germany).

Western and lectin blot

Vertical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), under both non-reducing and reducing conditions of 0.5 μg isolated IgA was performed on a SE 260 Mighty Small II Vertical Unit (Hoefer Inc., Holliston, MA, USA) [12]. Proteins on gels were visualized by Coomassie brilliant blue staining or transferred to nitrocellulose membrane using a semi-wet transfer method (Hoefer TE70X, Hoefer Inc.). The transfer efficacy was verified by Ponceau S staining.

Polyclonal goat anti-human IgA antibodies conjugated with peroxidase (Boster Biological Technology Co. Ltd, Pleasanton, CA, USA) were used to detect affinity-isolated IgA. Galactose and sialic acid expression on IgA molecules were examined by lectin blotting with *Ricinus communis* agglutinin I (RCA I) and *Sambucus nigra* agglutinin (SNA) both purchased from Vector Laboratories Inc., Newark, CA, USA.

Nitrocellulose membranes were incubated in 3% bovine serum albumin in Tris-buffered saline-Tween to block nonspecific binding. For Western blotting, the membranes were incubated with 10,000 × diluted anti-human IgA antibody. After the washing with Tris-buffered saline-Tween, the bands were visualized by chemiluminescence and imaged with ChemiDoc 2.0 (Bio-Rad Laboratories, Hercules, CA, USA). For lectin blotting, membranes were incubated with RCA I or SNA lectins (1 μg/ml and 2.5 μg/ml, respectively), followed by incubation with 1000-time diluted avidin-peroxidase (Vector Laboratories). The chemiluminescence intensity from both western and lectin blotting was quantified with Image Master Total Lab software (GE HealthCare, Chicago, IL, USA).

Sugar specificity of RCA I or SNA lectins was confirmed by inhibitory dot blot in the presence of 200 mM galactose and 400 mM N-acetylneuraminic acid (sialic acid). The concentration of inhibitory sugars was recommended by the manufacturer (Vector Laboratories).

Ethics: The investigations were approved by the the Military Medical Academy Ethics Committee (No. 17/2020) and the Institute for Blood Transfusion of Serbia Ethics Committee (No. 213/23)

RESULTS

Expression of galactose and sialic acid on total IgA isolated from the serum of healthy individuals

In this study, we first analyzed glycosylation of IgA isolated from a pooled serum sample of 100 healthy individuals, using affinity chromatography on peptide agarose M. Given the significant heterogeneity of immunoglobulin molecules and substantial individual variations in glycan expression on them [2, 3], this analysis of pooled serum samples provides more reliable information about the structure of immunoglobulin molecules in a particular population and minimizes physiological variations that may arise in a small subset of healthy individuals.

Following SDS-PAGE under non-reducing conditions, we observed a 160 kDa anti-human IgA antibody reactive protein, corresponding to IgA monomer (Figure 1.A1–A2). After SDS-PAGE under reducing conditions, proteins of 60 kDa and 25 kDa, corresponding to IgA α-heavy and light chains, were detected (Figure 1.B1). The presence of IgA α-heavy chain was confirmed by Western blot with anti-human IgA antibodies (Figure 1.B2).

The lectin blotting showed that isolated IgA monomer expressed both galactose and sialic acid (Figure 1.A3–A4), with expression primarily localized on the α-heavy chains (Figure 1.B3–B4). The expression of these two sugars on proteins corresponding to IgA-light chains was detectable, but weak. Besides IgA, "additional" RCA I or SNA reactive glycoproteins were detected in healthy human serum IgA

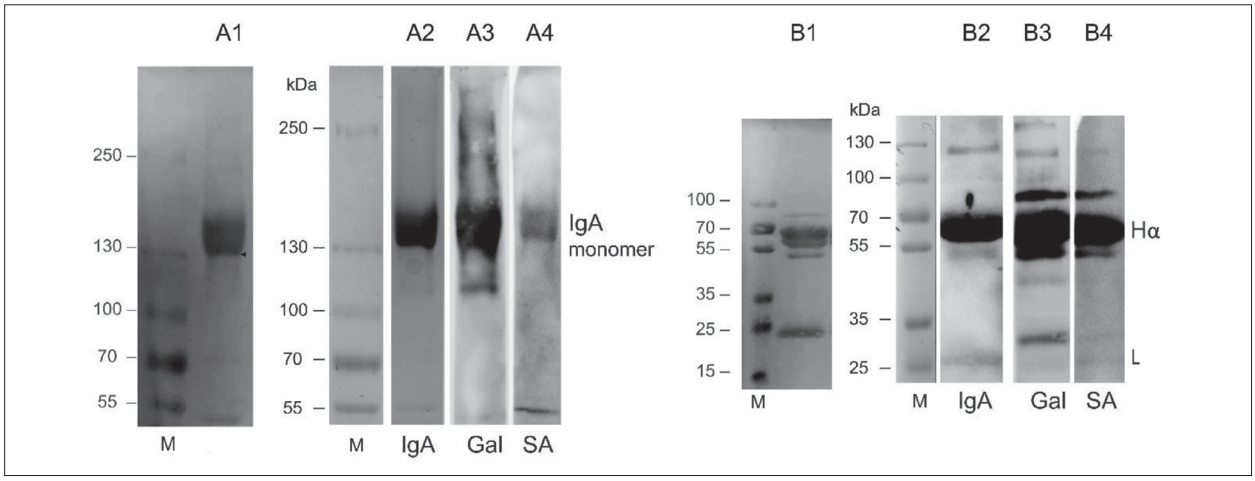


Figure 1. Expression of galactose and sialic acid on IgA from pooled healthy human serum; sodium dodecyl sulfate polyacrylamide gel electrophoresis under non-reducing (A1) or reducing conditions (B1); Western blot with anti-human IgA antibody (A2 and B2); lectin blot with galactose (Gal) specific *Ricinus communis* agglutinin I (RCA I) (A3 and B3); lectin blot with sialic acid (SA) specific *Sambucus nigra* agglutinin (SNA) (A4 and B4); M – molecular weight protein markers. Hα – IgA-heavy chains, L – IgA-light chains

Table 1. Paraprotein isotype, and demographic and clinical data of individuals with IgA multiple myeloma

Sample	Paraprotein	Age	Gender	β2micro (mg/L)	IgA (g/L)	IgM (g/L)	IgG (g/L)	κ/λ
1	mIgA1 κ	70	M	2.44	44.40	0.38	2.72	40.80
2	mIgA1 κ	50	M	5.92	63.10	< 0.18	1.61	0.02
3	mIgA1 κ	76	M	3.20	8.82	< 0.18	4.72	8.33
4	mIgA1 κ+κLC	67	F	1.93	18.90	< 0.18	4.84	11.60
5	mIgA1 κ	81	F	12.8	1.99	0.29	12.80	2.10
6	mIgA1 λ	88	M	2.65	3.96	2.17	7.20	0.84
7	mIgA1 κ	88	M	7.02	4.79	< 0.18	2.64	6.21
8	mIgA1 κ	81	F	3.53	20.70	0.27	5.96	11.00
9	mIgA1 λ	73	M	1.66	10.20	0.38	6.02	0.36
10	mIgA1 κ+κLC	69	F	3.64	34.40	< 0.18	7.76	6.60
11	mIgA1 κ	80	M	3.48	12.90	0.44	6.19	9.52
12	mIgA1 κ+κLC	81	F	7.49	25.20	< 0.18	5.25	12.00
13	mIgA1 κ	86	M	7.70	31.70	0.44	23.00	5.60
14	mIgAl λ	52	F	8.85	7.20	1.31	10.20	1.32
15	mIgAl λ	85	F	2.49	5.04	1.79	6.69	0.74
(Q1 – 25%)		69		2.49	5.04	0.17	4.72	0.84
Median		80		3.53	12.90	0.29	6.02	6.21
(Q3 – 75%)		85		7.49	31.70	0.44	7.76	11.00
Reference	Min			0.70	0.86	0.29	6.22	0.26
Values [17]	Max			1.80	4.69	2.60	15.10	1.65

M – male; F – female; LC – free light chains; Q1 – the first quartile, indicating the lower range (25%) of the distribution for the analyzed parameters; Q3 – the third quartile, 75% of the values observed are below this value; β2micro – β2-microglobulin; κ/λ – kappa/lambda light chain ratio

isolate (Figure 1 A3 and Figure 1 B2–B4). These proteins, which might represent serum proteins in complexes with IgA or nonspecifically bound proteins, were nor further analyzed.

Expression of galactose and sialic acid on total IgA isolated from the serum of individuals with IgA multiple myeloma

We analyzed galactose and sialic acid expression on IgA molecules isolated from 15 myeloma serum samples with confirmed IgA paraproteins. The patients' age, serum concentrations of β2-microglobulin, total serum IgA, IgM, and IgG, and the ratio of κ- and λ-light chains are given in Table 1. The concentration of IgA represents the sum of the concentrations of IgA paraproteins (i.e., monoclonal IgA) and residual polyclonal IgA. All 15 IgA paraproteins belonged to the IgA1 subclass.

We analyzed total, polyclonal and monoclonal, IgA molecules isolated by affinity chromatography from the serum of individuals with IgA multiple myeloma. Additional purification steps to separate monoclonal from most of residual polyclonal IgA molecules were not performed. SDS-PAGE under reducing conditions confirmed the presence of two protein bands of 60 kDa and 25 kDa, corresponded to IgA-heavy and light chains (Figure 2A). Western blot confirmed the fraction of 60 kDa as IgA-heavy chain (Figure 2B), and RCA I lectin blotting showed strong reaction with these heavy chains (Figure 2C). Conversely, SNA reaction, i.e., sialic acid expression, was weak and detected in 14 out of 15 isolates (Figure 2D). Light chains reacted strongly with RCA I in only three out of 15 isolated IgA, indicating that the expression of terminal galactose on 12 remaining IgA-light chains was below the detection limit of the method applied in our study. The results of the lectin blotting performed with SNA showed low, but detectable level of sialic acid on heavy chains in 14 and on light chains in 14 out of 15 IgA isolates. The specify of RCA I and SNA was tested with an inhibitory dot-blot (Figure 3), confirming that the observed RCA I and SNA reactivity originate from binding to terminal galactose and sialic acid on IgA oligosaccharides.

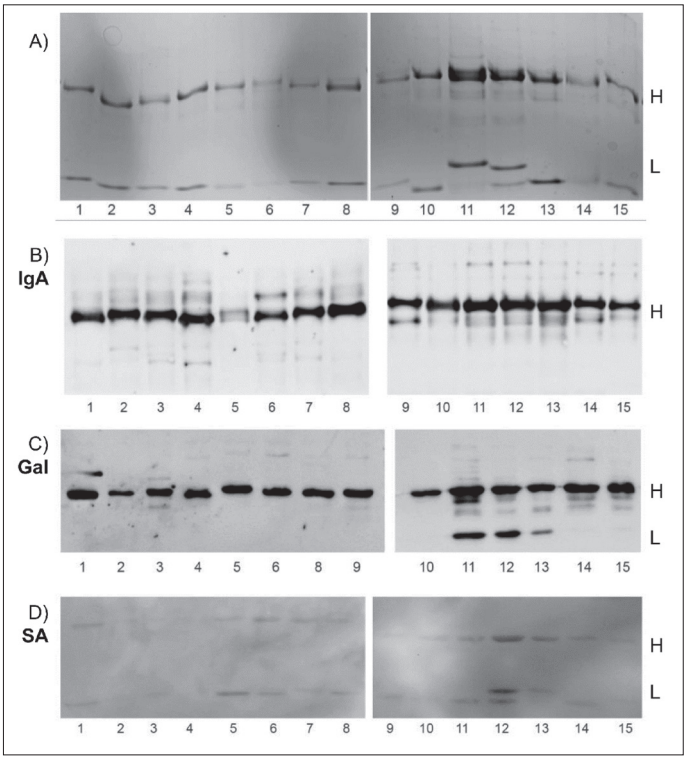


Figure 2. Expression of galactose (Gal) and sialic acid (SA) on light and heavy chains of total IgA isolated from the serum of individuals with IgA multiple myeloma; A – non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis; B – Western blot performed with anti-human IgA antibody; C – lectin blots performed with Gal specific *Ricinus communis* agglutinin I (RCA I); D –SA specific – *Sambucus nigra* agglutinin (SNA); H – heavy chains; L – light chains of IgA

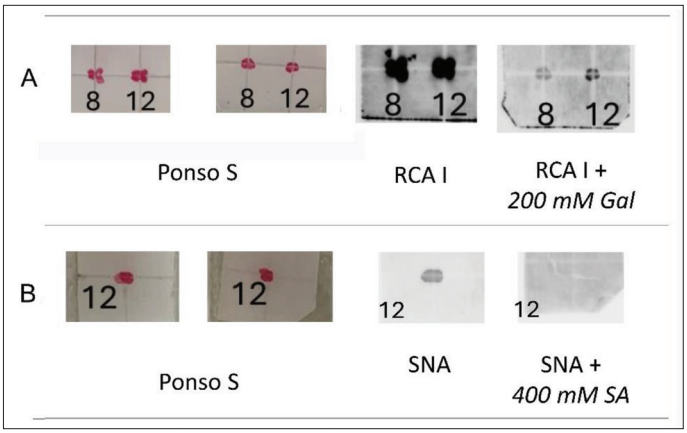


Figure 3. Confirming *Ricinus communis agglutinin* I (RCA I) and *Sambucus nigra* agglutinin (SNA) specificity with inhibitory dot blot; (A) – inhibition of RCA I binding to IgA isolates #8 and #12 with 200 mM galactose (Gal); (B) – inhibition of SNA binding to IgA isolate #12 (showing the highest SNA signal) with 400 mM sialic acid; Ponceau S – indicates the amount of dotted IgA

Densitometric analysis showed significant individual variations in galactose and sialic acid expression. The sialic acid expression levels were 67–97% lower in myeloma IgA compared to healthy individuals. Similarly, galactose expression was reduced by 12–63% in 13 out of 15 analyzed myeloma IgA-heavy chains relative to healthy controls (Figure 4). Compared to IgA from healthy individuals, the sialic acid to galactose expression ratio was lower on all analyzed myeloma IgA-heavy chains (Figure 4).

In contrast to IgA from pooled serum of healthy individuals (Figure 1.A1), sera from individuals with multiple myeloma were separated into two or more fractions after SDS-PAGE under non-reducing conditions, one with a molecular weight of 160 kDa, and other(s) with higher molecular weights (Figure 5). RCA I lectin blotting demonstrated that both monomeric and polymeric forms of myeloma IgA expressed galactose. The intensity of expression varies depending on the sample (Figure 4C and 4D). Sialic acid expression on either monomeric or polymeric forms of myeloma IgA was below the detection limit of SNA lectin blot performed after SDS-PAGE under non-reducing conditions.

DISCUSSION

We analyzed the expression of galactose and sialic acid on IgA molecules from 15 serum samples of myeloma patients with IgA paraproteins, all of which were of IgA1 subclass. The frequency of IgA2 paraproteins is lower than expected based on their concentration in healthy sera [13], so the absence of IgA2 paraproteins in our study was not unexpected. In 13 out of the 15 analyzed sera, the concentration of total IgA was higher than for individuals of similar age [14]. Immunoparesis, a hallmark of multiple myeloma characterized by the suppression of immunoglobulin isotypes [15], different from the paraprotein isotype was also observed.

For the purpose of analyzing the expression of galactose and sialic acid on IgA molecules, IgA was isolated from the pooled healthy human serum and sera of IgA myeloma patients using affinity chromatography on peptide agarose M. Peptide M binds with high affinity monomeric and dimeric human IgA serum, secretory IgA, both IgA1 and IgA2, and IgA in complexes with antigens [11] allowing isolation of all molecular forms of IgA molecules. With Peptide M affinity chromatography, we isolated both monoclonal and residual polyclonal IgA from myeloma serum samples. Additional purification steps for separation of monoclonal from most residual polyclonal IgA molecules, as previously described [7], were not performed in this study. However, we are aware that, in the case of IgG myeloma, it has been shown that the level of sialylation on residual polyclonal serum IgG may be higher than on the IgG paraprotein [16].

The results of our study showed intense reaction between the heavy chains of healthy IgA molecules and RCA I and SNA indicating a notable expression of galactose and sialic acid. All 15 analyzed myeloma IgA-heavy chains reacted with RCA I and 14 of them reacted with SNA but densitometric analysis showed lower expression of both galactose and sialic acid on heavy chains of myeloma IgA than on healthy IgA. Bosseboeuf et al. [7] also showed a reduced level of sialic acid in IgA of myeloma patients.

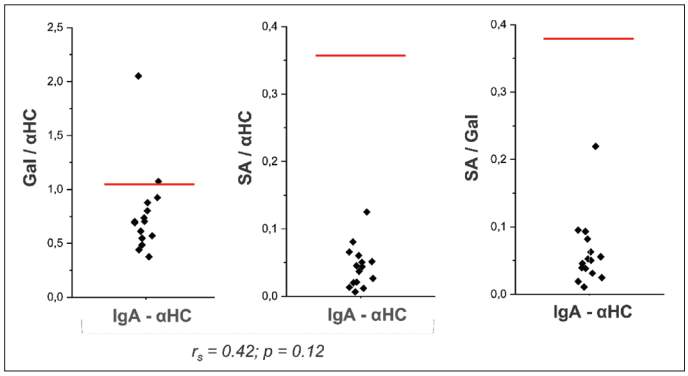
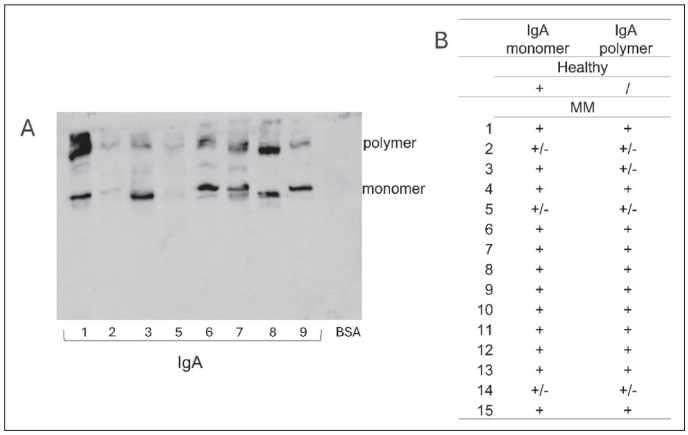


Figure 4. Densitometric quantification of the expression of galactose (Gal) and sialic acid (SA) on heavy chains (HC) of total serum IgA of individuals with IgA multiple myeloma and healthy individuals; the expression of monosaccharides presented as a ratio of chemiluminescence intensity (i.e., pixel count) in the lectin blot with *Ricinus communis* agglutinin I or *Sambucus nigra* agglutinin to the chemiluminescence intensity in the Western blot with anti-human IgA antibody



B

	IgA monomer	IgA polymer
	Healthy	
	+	/
	MM	
1	+	+
2	+/-	+/-
3	+	+/-
4	+	+
5	+/-	+/-
6	+	+
7	+	+
8	+	+
9	+	+
10	+	+
11	+	+
12	+	+
13	+	+
14	+/-	+/-
15	+	+

Figure 5. Expression of galactose (Gal) on monomeric and polymeric IgA isolated from the serum of individuals with IgA multiple myeloma (MM); A – *Ricinus communis* agglutinin I lectin blot; B – expression of Gal on monomeric and polymeric forms of IgA; (+) – strong expression; (+/-) – weak expression; BSA – bovine serum albumin – the assay negative control

Ding et al. [3] suggested that hyposialylation of IgA in multiple myeloma might affect the binding efficiency of IgA to FcαRI, consequently affecting IgA effector functions. Moreover, enzymatic desialylation of IgA1 of healthy people leads to proinflammatory profile of IgA1 [17]. It has long been known that reduced expression of galactose on O-linked glycans in the hinge region of heavy chain of IgA1 molecules is associated with IgA nephropathy. Renfrow et al. [5, 6] showed that decreased content of galactose in myeloma IgA1 is the result of aberrant galactosylation of O-linked glycans in the hinge region, with, similar to our results, reduced galactose content varying among the analyzed monoclonal IgA1 molecules. It remains unclear whether the immune complexes in IgA myeloma, like in IgA nephropathy [18], are formed between galactose-deficient O-glycans in IgA1 and anti-glycan IgG autoantibodies, leading to their deposition in the glomeruli and subsequent initiation of renal injury.

The expression of galactose and sialic acid on IgA-light chains was weak, and most likely reflected the rare carbohydrates immunoglobulin light chains occurring as the consequence of a variable region somatic mutation creating an Asn-[X≠Pro]-Ser/Thr [19] sequence to which carbohydrate group is attached to the asparagine residue of this triplet sequence [20]. Whether strong RCA I reactivity observed only on three IgA-light chains compared to week reactivity on other myeloma IgA-light chains reflects somatic mutation of glycan binding site within IgA variable regions, or germline-encoded variable region sequences containing glycan binding motive is not clear. Independently of their origin, glycosylation of monoclonal immunoglobulin light chains has been indicated as a potential risk for progression of monoclonal gammopathy of undetermined significance (MGUS) to myeloma and immunoglobulin light chain amyloidosis [21].

In this study, we confirmed that serum IgA of healthy individuals predominantly exists in monomeric form [22], with detectable expression of both galactose and sialic acid. In contrast, myeloma IgA existed in both monomeric and polymeric forms. Although polymeric IgA could represent complexes of IgA with some serum proteins, we consider it unlikely: non-covalent interactions between IgA and these proteins break down in the elution buffer used in affinity chromatography and non-reducing SDS-PAGE, but dimeric and polymeric forms of IgA are linked via J chains by covalent bonds that cannot be broken in the buffers. However, α1-antitrypsin covalently bound to IgA molecules forming IgA polymers was described in IgA myeloma sera [23], so the presence of such complexes cannot be entirely excluded. Polymeric forms of IgA are believed to be responsible for hyperviscosity syndrome [24] and represent predominant components of immune complexes detected in circulation and glomerular deposits in IgA nephropathy [25]. A similar role of polymeric IgA in multiple myeloma cannot be excluded. In IgA nephropathy, it has been shown that polymeric IgA significantly reduces the expression of FcαR on macrophages compared to monomeric IgA. This consequently decreases the binding of IgA1 and IgA1 immune complexes to FcαR and their degradation in macrophages, resulting in increased circulating levels of these complexes as well as proliferation of mesangial cells and glomerular sclerosis [26]. By RCA I lectin blotting, we demonstrated that both monomeric and polymeric myeloma IgA forms expressed galactose with sample-dependent intensity. Inversely, a very weak sialic acid expression was detected on heavy and light chains, and none on intact monomers or polymers of IgA. This "loss" of sialic acid expression might reflect methodological limitations: non-reducing SDS-PAGE cannot reach the "concentration" effect on proteins that SDS-PAGE under reducing conditions can. Therefore, the extremely small amount of sialic acid detected on separated heavy and light chains, which is now "divided" between the fractions of monomeric and polymeric IgA, may be less accessible to the SNA lectin due to the preserved structure of the complete molecules, and could not be detected by lectin blotting applied.

The pathogenic potential of differently galactosylated monomeric and polymeric myeloma IgA1 forms remains unclear. In IgA nephropathy, heavy chains of polymeric and monomeric IgA are differently N-glycosylated. Oligomannose-type glycan, an important signaling molecule for the activation of the complement system via the lectin pathway and the induction of inflammation in renal glomeruli, is exclusively present on polymeric IgA [27]. Pathogenic significance of complement activation via lectin pathway in IgA multiple myeloma remains obscure.

This study contributes to our understanding of glycosylation patterns of IgA in multiple myeloma, an area that remains less extensively characterized than IgG glycosylation in both physiological and pathological conditions. Despite the limited number of IgA myeloma samples and lack of clinical stratification by disease stage or treatment, due to the nature of obtaining blood samples, we demonstrate that galactose and sialic acid expression is markedly reduced on IgA-heavy chains from myeloma patients compared to healthy individuals. This finding is aligned with and extending previous reports [5, 6, 7], suggesting that altered glycosylation of myeloma IgA may carry functional relevance. Additionally, we believe that introducing clinical stratification by disease stage or treatment in a small cohort would risk misinterpretation. However, one should be also cautious when interpreting these results since both IgA subclasses are present in the peripheral blood of healthy individuals (90% IgA1 and 10% IgA2) [22] whereas analyzed paraproteins were classified in IgA1 subclass, and the level of residual IgA2 was not determined.

The use of lectin blotting in our analysis enabled us to examine the intact IgA glycoprotein, non-enzymatically digested, providing insights into the steric accessibility and composition of glycans on glycoproteins [28]. While this method does not distinguish between N- and O-linked monosaccharides or localize them to specific regions (e.g., CH2 antibodies, hinge, or variable regions of IgA-heavy chains), it offers a valuable insight into the functional availability of these sugars for interactions with immunoglobulin receptors or complement proteins, and their effector functions.

Immense heterogeneity of IgA glycoforms [3, 4] limits the ability to draw even generalized conclusions from our study or other studies [5, 6, 7] performed on relatively small number of samples. To provide a more comprehensive understanding of IgA glycosylation in multiple myeloma and pathogenic significance of IgA oligosacharides, the type of glycans (N- or O-linked) and precise binding site of IgA glycans (hinge region, Fc, or fragment antigen-binding region), the monosaccharides composition analysis of myeloma IgA oligosaccharides on a notably larger number of IgA isolated from peripheral blood of myeloma patients an advanced glycoproteomics with classical bio- and physicochemical approaches and with incorporated detailed clinical metadata is needed.

CONCLUSION

Myeloma IgA has altered glycosilation. Lectin blot analysis revealed that, compared to healthy human IgA, the expression of both galactose and sialic acid was reduced on heavy chains myeloma-derived IgA. The light chains of myeloma IgA were predominantly weakly sialylated; however, notable galactosylation of the light chains was observed in approximately one-fifth of the cases. In contrast to the predominantly monomeric IgA found in healthy individuals, myeloma sera contained both monomeric and polymeric forms. Lectin blotting detected galactose on both monomeric and polymeric myeloma IgA, although expression levels varied among patients. The sialic acid, however, was not detectable on either the monomeric or polymeric forms of myeloma IgA.

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REFERENCES

1. Visram A, Vaxman I, Saleh ASA, Parmar H, Dispenzieri A, Kapoor P, et al. Disease monitoring with quantitative serum IgA levels provides a more reliable response assessment in multiple myeloma patients. Leukemia. 2021;35(5):1428–37. [DOI: 10.1038/s41375-021-01180-x] [PMID: 33623138]
2. Abduh MS. An overview of multiple myeloma: A monoclonal plasma cell malignancy's diagnosis, management, and treatment modalities. Saudi J Biol Sci. 2024;31(2):103920. [DOI: 10.1016/j.sjbs.2023.103920] [PMID: 38283805]
3. Ding L, Chen X, Cheng H, Zhang T, Li Z. Advances in IgA glycosylation and its correlation with diseases. Front Chem. 2022;10:974854. [DOI: 10.3389/fchem.2022.974854] [PMID: 36238099]
4. Hansen AL, Reily C, Novak J, Renfrow MB. Immunoglobulin A Glycosylation and Its Role in Disease. Exp Suppl. 2021;112:433–77. [DOI: 10.1007/978-3-030-76912-3_14] [PMID: 34687019]
5. Renfrow MB, Cooper HJ, Tomana M, Kulhavy R, Hiki Y, Toma K, et al. Determination of aberrant O-glycosylation in the IgA1 hinge region by electron capture dissociation fourier transform-ion cyclotron resonance mass spectrometry. J Biol Chem. 2005;280(19):19136–45. [DOI: 10.1074/jbc.M411368200] [PMID: 15728186]
6. Renfrow MB, Mackay CL, Chalmers MJ, Julian BA, Mestecky J, Kilian M, et al. Analysis of O-glycan heterogeneity in IgA1 myeloma proteins by Fourier transform ion cyclotron resonance mass spectrometry: implications for IgA nephropathy. Anal Bioanal Chem. 2007;389(5):1397–407. [DOI: 10.1007/s00216-007-1500-z] [PMID: 17712550]
7. Bosseboeuf A, Seillier C, Mennesson N, Allain-Maillet S, Fourny M, Tallet A, et al. Analysis of the targets and glycosylation of monoclonal IgAs From MGUS and myeloma patients. Front Immunol. 2020;11:854. [DOI: 10.3389/fimmu.2020.00854] [PMID: 32536913]
8. Johansson BG. Agarose gel electrophoresis. Scand J Clin Lab Invest Suppl. 1972;124:7–19. [DOI: 10.3109/00365517209102747] [PMID: 4114361]
9. Sam-Yellowe TY. Exercise 10: Immunodiffusion and Immunoelectrophoresis. In: Immunology: Overview and Laboratory Manual. Cham: Springer; 2021. p. 311–8. [DOI: 10.1007/978-3-030-64686-8_34]

10. Djukić T, Drvenica I, Kovačić M, Milanović S, Majerič D, Šefik-Bukilica M, et al. Exploring the Link between Hydrodynamic Size and Immunoglobulins of Circulating Immune Complexes in Rheumatoid Arthritis. Int J Mol Sci. 2024;25(6):3138. [DOI: 10.3390/ijms25063138] [PMID: 38542112]
11. Sandin C, Linse S, Areschoug T, Woof JM, Reinholdt J, Lindahl G. Isolation and detection of human IgA using a streptococcal IgA-binding peptide. J Immunol. 2002;169(3):1357–64. [DOI: 10.4049/jimmunol.169.3.1357] [PMID: 12133959]
12. Djukić T, Drvenica I, Kovačić M, Minić R, Vučetić D, Majerič D, et al. Dynamic light scattering analysis of immune complexes in sera of rheumatoid arthritis patients. Anal Bioch. 2023;674:115194. [DOI: 10.1016/j.ab.2023.115194] [PMID: 37279816]
13. Lee AYS, Lin MW. Polymeric IgA paraprotein on agarose gel electrophoresis immunofixation identifies a unique subset of IgA myeloma patients. Clin Chim Acta. 2021;512:112–6. [DOI: 10.1016/j.cca.2020.10.031] [PMID: 33127346]
14. Khan SR, Chaker L, Ikram MA, Peeters RP, van Hagen PM, Dalm VASH. Determinants and Reference Ranges of Serum Immunoglobulins in Middle-Aged and Elderly Individuals: a Population-Based Study. J Clin Immunol. 2021;41(8):1902–14. [DOI: 10.1007/s10875-021-01120-5] [PMID: 34505230]
15. Chahin M, Branham Z, Fox A, Leurinda C, Keruakous AR. Clinical Considerations for Immunoparesis in Multiple Myeloma. Cancers (Basel). 2022;14(9):2278. [DOI: 10.3390/cancers14092278] [PMID: 35565407]
16. Mittermayr S, Bones J, Le GN, O'Gorman P. Characterization of myeloma paraprotein glycosylation reveals functional insights and increased sialylation of residual polyclonal antibodies. Blood. 2015;126:1775. [DOI: 10.1182/blood.V126.23.1775.1775]
17. Steffen U, Koeleman CA, Sokolova MV, Bang H, Kleyer A, Rech J, et al. IgA subclasses have different effector functions associated with distinct glycosylation profiles. Nat Commun. 2020;11(1):120. [DOI: 10.1038/s41467-019-13992-8] [PMID: 31913287]
18. Suzuki H, Yasutake J, Makita Y, Tanbo Y, Yamasaki K, Sofue T, et al. IgA nephropathy and IgA vasculitis with nephritis have a shared feature involving galactose-deficient IgA1-oriented pathogenesis. Kidney Int. 2018;93(3):700–5. [DOI: 10.1016/j.kint.2017.10.019] [PMID: 29329643]
19. Grigaite R, Iles JK, Harding S, Patel R, Wallis G, Iles RK. Degree of immunoglobulin kappa light chain glycosylation of anti-spike SARS CoV-2 antibodies correlates with COVID-19 severity [preprint]. medRxiv. 2023. [DOI: 10.1101/2023.01.06.23284259]
20. Singh G, Xu H, Bollag RJ. Monoclonal Light Chains in Multiple Myeloma: The Sinister Immunoglobulin. Int J Pathol Clin Res. 2022;8(2):134. [DOI: 10.23937/2469-5807/1510134] [PMID: 4193390]
21. Dispenzieri A, Larson DR, Rajkumar SV, Kyle RA, Kumar SK, Kourelis T, et al. N-glycosylation of monoclonal light chains on routine MASS-FIX testing is a risk factor for MGUS progression. Leukemia. 2020;34(10):2749–53. [DOI: 10.1038/s41375-020-0940-8] [PMID: 32594098]
22. Wang Y, Xiao J. Recent advances in the molecular understanding of immunoglobulin A FEBS J. 2024;291(16):3597–603. [DOI: 10.1111/febs.17089] [PMID: 38329005]
23. Laurell CB, Thulin E. Complexes in Human Plasma between α1-Antitrypsin and IgA, and α1-Antitrypsin and Fibrinogen. Scand J Immunol. 1975;4:7–12. [DOI: 10.1111/j.1365-3083.1975.tb03802.x]
24. Perez Rogers A, Estes M. Hyperviscosity Syndrome. 2023. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. [PMID: 30085505]
25. Hernando P, Egido J, de Nicolas R, Sancho J. Clinical significance of polymeric and monomeric IgA complexes in patients with IgA nephropathy. Am J Kidney Dis. 1986;8(6):410–6. [DOI: 10.1016/s0272-6386(86)80167-6] [PMID: 2949605]
26. Liu Y, Gong Y, Xu G. The role of mononuclear phagocyte system in IgA nephropathy: pathogenesis and prognosis. Front Immunol. 2023;14:1192941. [DOI: 10.3389/fimmu.2023.1192941] [PMID: 37529043]
27. Oortwijn BD, Roos A, Royle L, van Gijlswijk-Janssen DJ, Faber-Krol MC, Eijgenraam JW, et al. Differential glycosylation of polymeric and monomeric IgA: a possible role in glomerular inflammation in IgA nephropathy. J Am Soc Nephrol. 2006;17(12):3529–39. [DOI: 10.1681/ASN.2006040388] [PMID: 17050773]
28. Geyer H, Geyer R. Strategies for analysis of glycoprotein glycosylation. Biochim Biophys Acta. 2006;1764(12):1853–69. [DOI: 10.1016/j.bbapap.2006.10.007] [PMID: 17134948]

Експресија терминалне галактозе и сијалинске киселине на серумским *IgA* у мултиплом мијелому типа *IgA*

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САЖЕТАК

Увод/Циљ Сматра се да мање повољна прогноза *IgA* мултиплог мијелома у односу на друге форме мијелома може бити последица и промењене гликозилације *IgA* у овој болести. Анализирана је експресија терминалне галактозе и сијалинске киселине на гликанима *IgA* изолованим из серума болесника са мијеломом и упоређена је експресија ова два моносахарида на мијеломским *IgA* са њиховом експресијом на *IgA* серума здравих особа.

Методе *IgA* су изоловани из серума 15 особа са *IgA* мијеломом и збирног узорка серума 100 здравих особа афинитетном хроматографијом на пептиду М. Експресија терминалне галактозе и сијалинске киселине анализирана је лектинским блотом, на основу везивања аглутинина из *Ricinus communis I* и аглутинина из *Sambucus nigra* лектина.

Резултати Тешки ланци серумских *IgA* здравих особа и свих 15 особа са мијеломом експримирали су галактозу, док је сијалинска киселина детектована код здравих особа и 14 особа са мијеломом. Експресија галактозе и сијалинске киселине на тешким ланцима мијеломских *IgA* била је за 12–63% и 67–97% нижа у односу на њихову експресију на *IgA* здравих особа. Снажна галактозилација лаких ланаца детектована је само код три мијеломска *IgA*, док је слаба експресија сијалинске киселине на лаким ланцима детектована код 14 од 15 мијеломских *IgA*. *IgA* молекули здравих особа били су предоминантно у форми мономера и експримирали су и галактозу и сијалинску киселину. Мијеломски *IgA* били су присутни у форми и мономера и полимера. Обе форме експримирале су детектабилни ниво галактозе (иако је ниво експресије варирао међу узорцима), али не и сијалинске киселине.

Закључци Гликозилација *IgA* у мултиплом мијелому је измењена. У односу на здраву контролу, тешки ланци *IgA* у мултиплом мијелому имају нижу експресију и галактозе и сијалинске киселине, док су лаки ланци појединих мијеломских *IgA* снажно галактозиловани. Галактоза, али не и сијалинска киселина, присутна је у детектабилном нивоу на интактним мономерним и полимерним мијеломским *IgA*.

Кључне речи: мултипли мијелом; *IgA*; гликозилација; сијалинска киселина; галактоза

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Hypersensitivity pneumonitis – experiences in treatment so far and opening up new possibilities

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SUMMARY

Introduction/Objective Hypersensitivity pneumonitis is a diagnostic and therapeutic challenge. It occurs due to an excessive immune response to inhaling an antigen (bacteria, fungi, or protozoa) to which the patient was previously sensitized. This study analyzes the clinical characteristics of patients during a 10-year period (2014–2023) treated at the Institute for Pulmonary Diseases of Vojvodina.

Methods A retrospective study was conducted including 74 patients. The first phase included data collection, the second phase included statistical data processing, and the third included the description of the obtained statistical parameters and discussion.

Results The average age was 57.61 ± 12.6 years; 52.7% were men, and 56.8% were non-smokers. Most patients had one or more comorbidities (70.3%). There were only 27 (36.5%) patients hospitalized more than three times. Progression and regression occurred in 14 (28%) patients each, while cessation occurred in 22 (44%). Fatal outcomes occurred in 7 (9.5%). It was determined that in patients with three or more comorbidities, ≥ 3 hospitalizations (9; 34.6%) occurred more often than < 3 hospitalizations (2; 4.2%) (Fisher's exact test = 14.46; p = 0.04). By comparing the prognosis of the disease with the number of hospitalizations, we found a statistically significant association (Fisher's exact test = 13.95; p = 0.001). The progression of the disease in patients with ≥ 3 hospitalizations (10; 62.5%) occurred more often than in patients with < 3 hospitalizations (4; 11.8%).

Conclusion To obtain adequate data on the success of therapy and its impact on slowing down the disease, further monitoring of patients who are on antifibrotic therapy is necessary.

Keywords: antifibrotic therapy; extrinsic allergic alveolitis; progressive pulmonary fibrosis

INTRODUCTION

Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis, is one of the interstitial lung diseases (ILD). This disease occurs due to an exaggerated immune response to an antigen from the external environment, leading to symptoms that, in differential diagnosis, may resemble symptoms of acute lung infection or progressive irreversible lung damage, such as fibrosis [1, 2]. Numerous studies have assessed the prevalence of HP in various high-risk occupations. HP occurs most often among farmers and pigeon breeders. It can also occur in people employed in swimming pools, those exposed to contaminated air conditioners, employees exposed to tobacco, and others [3].

The most common symptoms are fever, myalgia, headache, cough, chest tightness, and dyspnea, usually occurring 4–12 hours after exposure to an antigen. The fibrotic form occurs due to prolonged exposure to the antigen, which initially causes inflammation and eventually leads to irreversible and often progressive interstitial fibrosis, which leads to respiratory failure [4].

According to the latest European Respiratory Association guidelines from 2022, HP is classified into fibrotic and non-fibrotic forms. A part of patients with chronic (fibrous) HP develop a progressive disease – progressive pulmonary fibrosis (PPF). For the diagnosis of PPF, at least two of the following three criteria are necessary: 1) worsening of respiratory symptoms; 2) pathophysiological criteria of disease progression (absolute decline in forced vital capacity (FVC) by ≥ 5% during one year of follow-up, or an absolute decrease in diffusing capacity of the lungs for carbon monoxide (DLCO) by ≥ 10% during one year of follow-up) and 3) radiological evidence of disease progression (one or more): increased volume of traction bronchiectasis and bronchiolectasia, increased volume of ground-glass opacity with traction bronchiectasis, new fine reticulations, increased volume or increased roughness of reticular abnormalities, new or increased volume of honeycombing, or loss of lung lobe volume [5].

Due to the lack of a diagnostic gold standard, diagnosing HP is not simple. It relies on numerous factors, such as history of exposure, clinical characteristics, antigen–antibody

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precipitation tests, radiological findings, lung function tests, and bronchological or surgical biopsies. Distinguishing HP from other ILDs can be challenging because HP's clinical, radiological, and pathohistological (PH) features are highly variable and overlap with those of other ILDs. The inflammatory disease may go unrecognized, while the fibrotic disease may be misdiagnosed as idiopathic pulmonary fibrosis [5, 6].

HP can be treated with pharmacological therapy, such as corticosteroids and immunosuppressants [mycophenolate mofetil (MMF), azathioprine, and monoclonal antibody rituximab]. Since 2022, nintedanib, a tyrosine kinase inhibitor, has been licensed in several countries to treat chronic fibrous ILD with a progressive phenotype. Another antifibrotic (pirfenidone) is still being investigated as a treatment for HP. Non-pharmacological therapy includes respiratory rehabilitation, continuous application of oxygen therapy, and lung transplantation. HP prevention includes avoiding allergens, getting regular vaccinations, avoiding smoking, and treating comorbidities [4, 7–10].

This paper aims to analyze the clinical characteristics of patients with HP treated at the Institute for Pulmonary Diseases of Vojvodina (IPDV) over a 10-year period.

METHODS

Participants and study design

The research was conducted in the form of a retrospective study, which included 74 patients with a diagnosis of HP who were treated at the Tuberculosis and Interstitial Lung Diseases Clinic of the Institute for Pulmonary Diseases of Vojvodina (IPDV) in Sremska Kamenica from January 1, 2014 to December 31, 2023. Data on patients diagnosed with HP found in the IPDV electronic database were gathered for the study's initial phase. The Integrated Health Information System (IHIS) was used to collect respondents' information. The following data were analyzed: sex, age, detection of disease (computed tomography, pathophysiology sample, multidisciplinary team decision), therapy, comorbidities, number of hospitalizations, smoking status, lung function parameter (FVC), and fatal outcome. By examining the respiratory function, the values taken for statistical processing were determined with the help of spirometry and gas analyses performed during the diagnosis and the last examination. The first finding before the therapy and the last finding where the patients used the therapy for a certain period were compared to assess the prognosis of HP according to the absolute change in FVC. During the data analyses, it was determined that a certain number of respondents lacked FVC parameters at the beginning or in the last finding, so in the final statistics, we had only 50 patients with valid FVC results. By comparing spirometric parameters, the absolute decline in FVC by ≥ 5% represented the progression of HP, while the absolute increase by ≥ 5% represented the regression of HP. If there was no decline or increase in FVC by ≥ 5%, it represented the cessation of the disease.

Analysis of data

Descriptive statistical methods, statistical hypothesis testing methods, and correlation methods were used for data analysis. With the help of descriptive statistical methods, standard statistics were performed, where the frequency was determined, measures of central tendency (arithmetic mean) were used for given parameters, and standard deviation was determined for variables where it was possible as a measure of variability, and relative numbers were used as indicators of structure. The non-parametric statistical analysis – χ^2 test and Fisher's exact test – were used to test statistical hypotheses. Using the φ correlation coefficient, the correlation between parameters was determined using Crosstabs. Hypotheses were tested at the level of statistical significance (α level) of 0.05. The results are shown in tables. Data were processed using IBM SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA).

Ethics: The study was approved by the relevant ethics committee of the IPDV (No. 33-III/1), and all data were used in accordance with applicable ethical guidelines and data protection regulations.

RESULTS

The research included 74 patients diagnosed with HP, with a slight male predominance. In the study, the average age of the patients was 57.61 ± 12.6 years; the youngest was 31 years old, and the oldest was 82 years old. The most common way of establishing the HP diagnosis was with the help of PH findings in more than half (58.1%) of the patients. More than two-thirds of patients received corticosteroids (CS) as therapy. There were 10 patients (14.1%) on dual therapy and one patient (1.4%) on triple therapy. The largest number of patients had one or more comorbidities (70.3%), with arterial hypertension being the most common and present in 34 (45.9%) patients, followed by 17 (23%) patients with cardiovascular diseases (CVD), and 6 (8.1%) patients with osteoporosis and diabetes mellitus type II (DM II). Non-smokers were dominant, with 42 (56.8%) patients, while only 13 (17.6%) patients were active smokers (Table 1). The mean number of smoked cigarettes per patient was 10.14 ± 14.91. The minimum number of cigarettes consumed daily was 10, and the maximum was 80.

Regarding hospitalizations, only 27 (36.5%) patients had more than three hospitalizations (Table 1). No statistically significant association was found when comparing the number of hospitalizations with either sex (Fisher's exact test = 3.72; p = 0.09) or age (Fisher's exact test = 2.67; p = 0.14). Analysis of the relationship between smoking status and the number of hospitalizations also did not show a statistically significant association. A statistically significant moderate positive correlation (φ = 0.45; p = 0.04) was found between the number of comorbidities and hospitalizations (Fisher's exact test = 14.46; p = 0.04). With the help of the Z test, it was determined that in patients without comorbidities, ≥ 3 hospitalizations (18; 37.5%) occurred more often than < 3 hospitalizations (3; 11.5%).

Table 1. Frequency and percentage of patients with hypersensitivity pneumonitis across different variables

Variables		n (%)
Sex	Male	39 (52.7)
	Female	35 (47.3)
Detection of disease	Pathohistology	43 (58.1)
	CT	19 (25.7)
	Multidisciplinary team	12 (16.2)
Therapy	CS	50 (70.4)
	AZ	7 (9.9)
	AF	3 (4.2)
	CS + AZ	6 (8.5)
	CS + AF	3 (4.2)
	AZ + AF	1 (1.4)
	CS + AZ + AF	1 (1.4)
Comorbidities	0	22 (29.7)
	1	20 (27)
	2	21 (28.4)
	≥ 3	11 (14.9)
Smoking status	Smokers	13 (17.6)
	Ex smokers	19 (25.6)
	Non-smokers	42 (56.8)
Hospitalization	< 3	47 (63.5)
	≥ 3	27 (36.5)
Prognosis of HP	Progression	14 (28)
	Cessation	22 (44)
	Regression	14 (28)
Fatal outcome	Yes	7 (9.5)
	No	67 (90.5)

CS – corticosteroid therapy; AZ – azathioprine; AF – antifibrotic therapy; HP – hypersensitivity pneumonitis

Table 2. Number of hospitalizations and death outcomes depending on sex, age, smoking status, number of comorbidities and pronosis of hypersensitivity pneumonitis

Variables		Hospitalizations		p	Fatal outcome		p
		< 3	≥ 3		Yes	No	
Sex	Women	26 (54.2%)	8 (30.8%)	0.09	1 (14.3%)	33 (49.3%)	0.12
	Men	22 (45.8%)	18 (69.2%)		6 (85.7%)	34 (50.7%)	
Age	< 60 years	28 (58.3%)	10 (38.5%)	0.14	2 (28.6%)	36 (53.7%)	0.26
	≥ 60 years	20 (41.7%)	16 (61.5%)		5 (71.4)%	31 (46.3%)	
Smoking status	Smokers	13 (27.1%)	2 (7.7%)	0.14	2 (28.6%)	13 (19.4%)	0.72
	Non-smokers	24 (50%)	16 (61.5%)		4 (57.1%)	36 (53.7%)	
	Ex-smokers	11 (22.9%)	8 (30.8%)		1 (14.3%)	18 (26.9%)	
Comorbidities	No comorbidities	18 (37.5%)	3 (11.5%)	0.04*	1 (14.3%)	20 (29.9%)	0.08
	1	13 (27.1%)	8 (30.8%)		0 (0%)	21 (31.2%)	
	2	15 (31.3%)	6 (23.1%)		3 (42.9%)	18 (26.9%)	
	≥ 3	2 (4.2%)	9 (34.6%)		3 (42.9%)	8 (12%)	
Prognosis of HP	Progression	4 (11.8%)	10 (62.5%)	0.001*	2 (66.7%)	12 (25.5%)	0.45
	Cessation	18 (52.9%)	4 (25%)		1 (33.3%)	21 (44.7%)	
	Regression	12 (35.3%)	2 (12.5%)		0 (0%)	14 (29.8%)	

*p < 0.05

Also, in patients with three or more comorbidities, ≥ 3 hospitalizations (9; 34.6%) occurred more often than < 3 hospitalizations (2; 4.2%) (Fisher's exact test = 4.02; p = 0.14) (Table 2).

Most of the patients with HP were in cessation (22; 44%), while the same number of patients were in progression and regression (14; 28%) (Table 1). By comparing the prognosis of HP to the number of hospitalizations, we found a statistically significant association (Fisher's exact test = 13.95; p = 0.001). The correlation coefficient showed a moderate positive correlation between the prognosis of HP and the number of hospitalizations (φ = 0.53; p = 0.001). The progression of HP in patients with ≥ 3 hospitalizations (10; 62.5%) occurred more often than in patients with < 3 hospitalizations (4; 11.8%). The number of patients with cessation and regression of HP did not differ between patients with < 3 and ≥ 3 hospitalizations (Table 2).

No statistically significant association was found when comparing fatal outcome with gender (Fisher's exact test = 3.12; p = 0.12), age (Fisher's exact test = 1.62; p = 0.26), smoking status (Fisher's exact test = 0.78; p = 0.72), number of comorbidities (Fisher's exact test = 7.92; p = 0.08) (Table 2) or prognosis of HP (Fisher's exact test = 2.18; p = 0.45) (Table 2).

DISCUSSION

In this retrospective study, we analyzed data from 74 patients diagnosed with HP over a ten-year period from January 2014 to December 2023.

Our results show that the higher frequency of HP was in male patients (52.7%), and the average age was 57.61 ± 12.60 years. Nishida et al. [11] concluded that HP was also more common in men (50.41%), and the average age of the subjects was 63 years, similar to our study. Prior et al. [12] found similar data, showing that HP was more common in male patients (53.6%) and that the mean age was 63 ± 13.3 years.

Our study showed that there were 42 non-smokers (56.8%), 19 of them ended their active smoking status (ex-smokers) (25.7%), while there were 13 current smokers (17.6%). The data found in the study conducted by Prior et al. [12] indicate that 50.2% of respondents were non-smokers. In the research by Selman et al. [13], it is highlighted as an interesting aspect that HP is less common in cigarette smokers. They believe nicotine inhibits the activation of macrophages, the proliferation of lymphocytes, and their function, but this information does not apply to former smokers. They also state that if HP occurs in smokers, the clinical picture and prognosis are worse than in non-smokers. From 80% to 95% of patients with HP do not use tobacco. Creamer and Barratt [14] and Alexandre et al. [15] reported that HP is more common in non-smokers than in active smokers.

The largest number of patients from our study had no comorbidities associated with HP (29.7%); 27% had one comorbidity, 28.4% had two comorbidities, and 14.9% had three or more. The most common comorbidities were arterial hypertension (45.9%), CVD (23%), osteoporosis, and DM II (8.1%). Wälscher et al. [16] identified that the most frequent number of comorbidities in HP patients was 3.

Of 211 patients, 11% had no comorbidities, 58% had 1–3 comorbidities, and 31% had ≥ 4 comorbidities. The most common comorbidities found were cardiovascular (65%). According to the study by Prior et al. [12], the most common comorbidities were arterial hypertension (55.5%), DM II (20.4%), ischemic heart disease (17.5%), obstructive lung disease (9.5%), and pulmonary hypertension (9.5%) [12, 16].

The most diagnosed cases in our research were with the help of PH (43/74; 58.1%). In their study, Noh et al. [17] concluded that HP was diagnosed with biopsy and PH findings in significantly fewer patients (40%), and Lacasse et al. [18] concluded that HP was diagnosed based on PH findings in 37%. In the study by Casal et al. [19], 12.4% of patients, as in our case, were diagnosed based on the clinical presentation. Koyuncu et al. [20] stated that the PH diagnosis of HP was made in 43 (55.1%) patients and that the multidisciplinary team decided on the diagnosis of HP in 19 (24.7%) patients. In the study by Adams et al. [21], 85.7% of the group underwent an invasive technique (bronchoalveolar lavage, transbronchial biopsy, and/or surgical lung biopsy) to confirm the diagnosis of HP [17–21].

Improving diagnostic accuracy for HP is based on a multidisciplinary approach. This includes detailed anamnesis with environmental and occupational exposure history, high-resolution CT imaging, serological testing for antigen-specific antibodies, which is not a diagnostic standard in our country, unfortunately, and histopathology sampling when invasive diagnosis is needed. The use of diagnostic algorithms that integrate radiological, clinical, and pathological findings is essential in differentiating HP from other interstitial lung diseases, like idiopathic pulmonary fibrosis.

Impaired lung function in patients with HP plays a critical role in determining the severity of the disease. However, it does not differ from other interstitial lung diseases. Restrictive lung ventilation disorder is the most common pathological finding in these patients. In our research, in 22 (44%) patients, there was no significant change in FVC; in 14 (28%) patients there was a significant increase in FVC by ≥ 5%; and in another 14 (28%) patients there was a significant decline in FVC value by ≥ 5%. In the study conducted by Macaluso et al. [22], the initial average FVC value in the participants was 67.5%. A decline of ≥ 5% in FVC during the first year was observed in 45 patients (31%).

In recent years, MMF has been used in the treatment of HP. Casal et al. [23] conducted a study in which after a one-year treatment with MMF, FVC stabilized and DLCO improved significantly. In the study by Okuda et al. [24], the immunosuppressant-with-prednisolone group's mean change in FVC in the 12 months before therapy was substantially lower than that of the immunosuppressant-naive prednisolone group. In an analysis conducted by Kaneko et al. [25], the yearly FVC drop in the PPF group reduced from -11.5% before therapy to -4.2% in the first year following treatment ($p < 0.001$). There was no significant difference between the annual FVC change before and after therapy in the non-PPF group (1.6% *vs.* -1.7%, $p = 0.065$).

We found that the largest number of patients were treated with corticosteroids (50/74; 70.64%), followed by patients who used azathioprine (7/74; 9.9%), and in third place were patients on antifibrotic drugs (nintedanib) (3/74; 4.2%). The therapeutic indication for antifibrotic therapy for progressive forms of HP was expanded in Serbia in 2022, and due to this fact, the number of patients receiving this therapy is small. In their study, Salisbury et al. [26] noted that the most common forms of HP treatment were corticosteroids and azathioprine. Wijsenbeek et al. [27] monitored the effects of nintedanib in patients with progressive pulmonary fibrosis (PPF) using the Living with Pulmonary Fibrosis (L-PF) questionnaire. Based on the assessment of L-PF questionnaire scores, nintedanib reduced the worsening of symptoms of dyspnea, fatigue, and cough during the 52-week study in patients with PPF.

Although lung transplantation is not possible in our country, it is a treatment for patients with HP in developed countries. In the University Hospital of Munich, in the last 30 years, there were a total of 1054 lung transplantations performed, and the best five-year survival rate was observed in patients with lymphangioleiomyomatosis (LAM) and HP [28].

Our study found a statistically significant correlation between the number of comorbidities and hospitalizations. No statistically significant association was found between the death outcome and the number of comorbidities. Prior et al. [12] state that there was a statistically significant association between the number and type of comorbidities with the death outcome and increased number of hospitalizations. The highest number of deaths was in the group of patients with cardiovascular comorbidities. A combination of better patient education, earlier diagnosis, and more proactive disease care is probably needed to lower hospitalization rates among HP patients. Broader screening for environmental exposures, prompt therapy beginning after diagnosis, and multidisciplinary team participation are a few possible interventions. Additionally, improving patient access to social support services, regular follow-up, and pulmonary rehabilitation can help manage comorbidities and reduce illness exacerbations, which may minimize the frequency of hospitalizations. Wälscher et al. [16] report that deceased patients had more comorbidities than survivors. A study by Schwarzkopf et al. [29] reported that comorbidities had a clinically significant adverse effect on survival that was more pronounced in the case of untreated comorbidities. In our study, the reason for the cause-and-effect relationship between a higher number of comorbidities and a lower number of hospitalizations is not known. We can only assume that the patients received adequate therapy for associated diseases and controlled them well.

Fatal outcomes were observed in seven (9.5%) of our patients. In a study conducted by Gonnelli et al. [30], the five-year survival rate after diagnosis of HP was 66%.

The limitations of this study are: data on the pulmonary function parameter (FVC) were not available in all patients; diffusion capacity (DLCO) values were not available in most patients; therefore, the DLCO parameter was not extracted from the system in this study. The primary reason for the low number of patients in our cohort who

received antifibrotic medication is that antifibrotics were only recently authorized for use in Serbia, specifically in 2022, for progressive types of HP. As a result, only a small number of patients had access to these medications during the study period.

CONCLUSION

With the introduction of new treatment protocols for the use of antifibrotic drugs, further studies are necessary to include a more extended period and a larger number of patients on antifibrotic therapy to obtain more complete data on the success of the therapy and its effect on disease regression. Treatment will become increasingly complex, with new therapeutic options and the possibility of daily progress, taking into account pharmacological and non-pharmacological forms of therapy and a multidisciplinary approach. The future of patients with PPF is realized through the joint effort of each medical worker who treats these patients and the patients themselves.

Conflict of interest: None declared.

REFERENCES

1. Reynolds C, Feary J, Cullinan P. Occupational contributions to interstitial lung disease. Clin Chest Med. 2020;41(4):697–707. [DOI: 10.1016/j.ccm.2020.08.015] [PMID: 33153688]
2. Walkoff L, Hobbs S. Chest imaging in the diagnosis of occupational lung diseases. Clin Chest Med. 2020;41(4):581–603. [DOI: 10.1016/j.ccm.2020.08.007] [PMID: 33153681]
3. Quirce S, Vandenplas O, Campo P, Cruz MJ, de Blay F, Koschel D, et al. Occupational hypersensitivity pneumonitis: an EAACI position paper. Allergy. 2016;71(6):765–79. [DOI: 10.1111/all.12866] [PMID: 26913451]
4. Hamblin M, Prosch H, Vašáková M. Diagnosis, course and management of hypersensitivity pneumonitis. Eur Respir Rev. 2022;31(163):210169. [DOI: 10.1183/16000617.0169-2021] [PMID: 35140104]
5. Raghu G, Remy-Jardin M, Richeldi L, Thomson CC, Inoue Y, Johkoh T, et al. Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT guideline. Am J Respir Crit Care Med. 2022;205(9):e18–e47. [DOI: 10.1164/rccm.202202-0399ST] [PMID: 35486072]
6. Kang J, Kim YJ, Choe J, Chae EJ, Song JW. Acute exacerbation of fibrotic hypersensitivity pneumonitis: incidence and outcomes. Respir Res. 2021;22(1):152. [DOI: 10.1186/s12931-021-01748-2] [PMID: 34016104]
7. Barnes H, Troy L, Lee CT, Sperling A, Strek M, Glaspole I. Hypersensitivity pneumonitis: current concepts in pathogenesis, diagnosis, and treatment. Allergy. 2022;77(2):442–53. [DOI: 10.1111/all.15017] [PMID: 34293188]
8. Adegunsoye A, Oldham JM, Pérez ER, Hamblin M, Patel N, Tener M, et al. Outcomes of immunosuppressive therapy in chronic hypersensitivity pneumonitis. ERJ Open Res. 2017;3(3):00016–2017. [DOI: 10.1183/23120541.00016-2017] [PMID: 28845429]
9. Mateos-Toledo H, Mejía-Ávila M, Rodríguez-Barreto Ó, Mejía-Hurtado JG, Rojas-Serrano J, Estrada A, et al. An open-label study with pirfenidone on chronic hypersensitivity pneumonitis. Arch Bronconeumol. 2020;56(3):163–9. [DOI: 10.1016/j.arbres.2019.08.019] [PMID: 31784348]
10. Ejima M, Okamoto T, Suzuki T, Miyazaki Y. Role of serum surfactant protein-D as a prognostic predictor in fibrotic hypersensitivity pneumonitis. Respir Investig. 2022;60(3):369–78. [DOI: 10.1016/j.resinv.2021.12.003] [PMID: 34998715]
11. Nishida T, Kawate E, Ishiguro T, Kanauchi T, Shimizu Y, Takayanagi N. Antigen avoidance and outcome of non-fibrotic and fibrotic hypersensitivity pneumonitis. ERJ Open Res. 2022;8(1):00474–2021. [DOI: 10.1183/23120541.00474-2021] [PMID: 35141326]
12. Prior TS, Wälscher J, Gross B, Bendstrup E, Kreuter M. Clusters of comorbidities in fibrotic hypersensitivity pneumonitis. Respir Res. 2022;23(1):368. [DOI: 10.1186/s12931-022-02291-4] [PMID: 36539821]
13. Selman M, Pardo A, King TE Jr. Hypersensitivity pneumonitis: insights in diagnosis and pathobiology. Am J Respir Crit Care Med. 2012;186(4):314–24. [DOI: 10.1164/rccm.201203-0513CI] [PMID: 22679012]
14. Creamer AW, Barratt SL. Prognostic factors in chronic hypersensitivity pneumonitis. Eur Respir Rev. 2020;29(156):190167. [DOI: 10.1183/16000617.0167-2019] [PMID: 32414744]
15. Alexandre AT, Martins N, Raimundo S, Melo N, Mota PC, Bastos HN, et al. Impact of azathioprine use in chronic hypersensitivity pneumonitis patients. Pulm Pharmacol Ther. 2020;60:101878. [DOI: 10.1016/j.pupt.2019.101878] [PMID: 31862300]
16. Wälscher J, Gross B, Morisset J, Johannson KA, Vašáková M, Bruhwyler J, et al. Comorbidities and survival in patients with chronic hypersensitivity pneumonitis. Respir Res. 2020;21(1):12. [DOI: 10.1186/s12931-020-1283-8] [PMID: 31918716]
17. Noh S, Yadav R, Li M, Wang X, Sahoo D, Culver DA, et al. Use of leflunomide in patients with chronic hypersensitivity pneumonitis. BMC Pulm Med. 2020;20(1):199. [DOI: 10.1186/s12890-020-01227-2] [PMID: 32693781]
18. Lacasse Y, Selman M, Costabel U, Dalphin JC, Ando M, Morell F, et al. Clinical diagnosis of hypersensitivity pneumonitis. Am J Respir Crit Care Med. 2003;168(8):952–8. [DOI: 10.1164/rccm.200301-137OC] [PMID: 12842854]
19. Casal A, Suárez-Antelo J, Riveiro V, Ferreiro L, Rodríguez-García C, de Alegría AM, et al. Hypersensitivity pneumonitis: application of a new diagnostic algorithm to a time series of the disease. Expert Rev Respir Med. 2024;18(3–4):237–43. [DOI: 10.1080/17476348.2024.2358939] [PMID: 38775489]
20. Koyuncu A, Sarı G, Şimşek C. Evaluation of cases with hypersensitivity pneumonia: 10-year analysis. Clin Respir J. 2023;17(4):329–38. [DOI: 10.1111/crj.13598] [PMID: 36780898]
21. Adams TN, Redlich CA, Glazer CS, Gulati M. Hypersensitivity pneumonitis associated with home mold exposure: a retrospective cohort analysis. PLoS One. 2025;20(5):e0323093. [DOI: 10.1371/journal.pone.0323093] [PMID: 40338891]
22. Macaluso C, Boccabella C, Kokosi M, Sivarasan N, Kouranos V, George PM, et al. Short-term lung function changes predict mortality in patients with fibrotic hypersensitivity pneumonitis. Respirology. 2022;27(3):202–8. [DOI: 10.1111/resp.14204] [PMID: 35023231]
23. Casal A, Suárez-Antelo J, Gude F, Lado-Baleato Ó, Otero B, Toubes ME, et al. Use of mycophenolate mofetil for the treatment of fibrotic hypersensitivity pneumonitis. Am J Med Sci. 2025;369(1):24–34. [DOI: 10.1016/j.amjms.2024.07.021] [PMID: 39009283]
24. Okuda R, Takemura T, Misumi T, Komatsu S, Hagiwara E, Ogura T. Effects of immunosuppressants in patients with mild fibrotic hypersensitivity pneumonitis. Respir Investig. 2025;63(1):13–9. [DOI: 10.1016/j.resinv.2024.11.011] [PMID: 39612544]
25. Kaneko T, Okuda R, Takemura T, Iwasawa T, Haga S, Takeda Y, et al. Antifibrotic agents in progressive and non-progressive pulmonary fibrosis of fibrotic hypersensitivity pneumonitis. Respir Investig. 2025;63(5):737–43. [DOI: 10.1016/j.resinv.2025.06.004] [PMID: 40541130]
26. Salisbury ML, Myers JL, Belloli EA, Kazerooni EA, Martinez FJ, Flaherty KR. Diagnosis and treatment of fibrotic hypersensitivity pneumonia: where we stand and where we need to go. Am J Respir Crit Care Med. 2017;196(6):690–9. [DOI: 10.1164/rccm.201608-1675PP] [PMID: 28002680]
27. Wijsenbeek M, Kreuter M, Olson A, Fischer A, Bendstrup E, Wells CD, et al. Progressive fibrosing interstitial lung diseases: current practice in diagnosis and management. Curr Med Res Opin. 2019;35(11):2015–24. [DOI: 10.1080/03007995.2019.1647040] [PMID: 31328965]

28. Vorstandlechner M, Schneider CP, Fertmann JM, Michel S, Kneidinger N, Walter J, et al. Thirty years of lung transplantation: development of postoperative outcome and survival over three decades. J Thorac Dis. 2024;16(12):8513–27. [DOI: 10.21037/jtd-24-326] [PMID: 39831218]
29. Schwarzkopf L, Witt S, Waelscher J, Polke M, Kreuter M. Associations between comorbidities, their treatment and survival in patients with interstitial lung diseases – a claims data analysis. Respir Res. 2018;19(1):73. [DOI: 10.1186/s12931-018-0769-0] [PMID: 29695236]
30. Gonnelli F, Eleangovan N, Smith U, Heatley H, Navarantam V, Corte TJ, et al. Incidence and survival of interstitial lung diseases in the UK, 2010–2019. ERJ Open Res. 2025;11(2):00823–2024. [DOI: 10.1183/23120541.00823-2024] [PMID: 40040895]

Хиперсензитивни пнеумонитис – досадашња искуства у лечењу и отварање нових могућности

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САЖЕТАК

Увод/Циљ Хиперсензитивни пнеумонитис представља дијагностички и терапијски изазов. Настаје као последица претераног имунолошког одговора на удисање антигена (бактерија, гљивица или протозоа) на које је болесник претходно сензибилисан.

Циљ ове студије био је да се анализирају клиничке карактеристике болесника лечених у Институту за плућне болести Војводине током 10 година (2014–2023).

Методе Спроведена је ретроспективна студија са 74 болесника. Прва фаза истраживања обухватала је прикупљање података о болесницима, друга фаза статистичку обраду података, а трећа фаза опис добијених статистичких параметара и дискусију.

Резултати Просечна старост болесника била је 57,61 ± 12,6 година; 52,7% су били мушкарци, а 56,8% непушачи. Највише болесника имало је један или више коморбидитета (70,3%). Само 27 (36%) болесника било је хоспитализовано више од три пута. До прогресије и регресије дошло је код 14 (28%) болесника, док је стагнација потврђена код 22 (44%) болесника. Фатални исход се десио код седам (9,5%) болесника. Утврђено је да се код болесника са три или више коморбидитета чешће јављају ≥ 3 хоспитализације (9; 34,6%) него < 3 хоспитализације (2; 4,2%) (Фишеров тест = 14,46; $p = 0,04$). Упоређујући прогнозу болести са бројем хоспитализација, установљена је статистички значајна повезаност (Фишеров тест = 13,95; $p = 0,001$). Прогресија болести код болесника са ≥ 3 хоспитализације (10; 62,5%) чешћа је него код болесника са < 3 хоспитализације (4; 11,8%).

Закључак За добијање адекватних података о успешности терапије и њеном утицају на
успоравање болести неопходно је даље праћење болесника који су на антифибротској терапији.

Кључне речи: антифибротска терапија; екстринзични алергијски алвеолитис; прогресивна плућна фиброза

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of topical application of tranexamic acid on the occurrence of postoperative hematoma after inguinal hernia repair using the Lichtenstein technique

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SUMMARY

Introduction/Objective The objective of the study was to determine whether the local application of tranexamic acid (TXA) by pouring over the surgical wound reduces the incidence of postoperative hematoma and the occurrence of postoperative bleeding in the wound following inguinal hernia repair using the Lichtenstein technique.

Methods We conducted a prospective, randomized, double-blind clinical study in the period from June 2024 to February 2025. One hundred and twenty patients were divided into two groups, one group of patients who received TXA during surgery, and the other group where a placebo was administered. Subsequently, the groups were compared and analyzed by sex and age structure, the frequency of postoperative hematoma, and the pre- and postoperative levels of erythrocytes, hemoglobin, and platelets were recorded.

Results The results indicate that in the group where TXA was administered, postoperative hematoma occurred statistically significantly less often than in the group with a placebo ($p < 0.05$). The frequency of postoperative hematoma in the group of patients who were treated with TXA was 3.8%, while the frequency in the group of patients who were treated with a placebo was 13%. There was a statistically significant difference in the levels of platelets before and after surgery in the compared groups.

Conclusion We found that the topical application of TXA reduces the occurrence of postoperative hematoma, and thus the occurrence of postoperative bleeding, as well as reduces the postoperative drop in platelets.

Keywords: inguinal hernia repair; tranexamic acid; hematoma; hemoglobin; erythrocytes; platelets

INTRODUCTION

Postoperative bleeding is one of the most common complications of surgical procedures. According to some reports, more than 300 million patients undergo surgery annually [1]. Bleeding can be merely a clinical sign, without any consequences, but it can also be significant, with varying consequences, and it occurs within the first 7 days in 77.7% of cases [2]. This affects the patient's morbidity and mortality, quality of life, and speed of recovery. According to a study conducted by Roshanov, mortality occurs in 5.8% of cases among patients with postoperative bleeding, excluding cardiac surgery patients [3]. There are various definitions of postoperative hemorrhage; however, our scope of interest is bleeding in the wound after inguinal hernia surgery using the Lichtenstein technique, manifesting as hematoma, i.e., bruising and/or swelling and out-of-range blood test results. The drug that is standardly used to prevent or stop bleeding is TXA. This agent is routinely used intravenously and orally, according to the manufacturer's recommendations, and has its own indications and contraindications [4, 5].

Systemic administration of TXA has shown many advantages, and most commonly cited are reduced postoperative bleeding and a reduced need for blood transfusion [6–9]. However, the idea of using TXA in the form of a local application emerged because there is unclear data on adverse effects since the drug manufacturer suggests avoiding the drug in certain patient groups, as a potential health risk [4, 5]. Several studies, systematic reviews, and meta-analyses addressed this topic, including the local application of TXA [10, 11–16]. So far, local application of TXA has been applied in various fields of surgery, such as otorhinolaryngology, maxillofacial surgery, orthopedics, breast surgery, spinal surgery, thoracic surgery, urology, and plastic surgery. However, some studies have described side effects following the local application of TXA, such as impaired wound healing, a larger amount of seroma after breast mastectomy and axillary dissection, and poor tendon healing [17, 18, 19]. Two studies also described that it enhances bone healing [20, 21]. Only one study investigated a similar topic regarding the local application of TXA during inguinal hernia repair, with a small number of patients at increased risk of bleeding due to

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the medications they used [16]. For this reason, our study aimed to test whether the local, more precisely topical application of TXA affects the reduction in the frequency of postoperative hematoma, i.e., bleeding after inguinal hernia repair using the Lichtenstein technique.

METHODS

A prospective, randomized, double-blinded study was conducted in the period from June 2024 to February 2025. The sample size consisted of 120 patients, divided into two groups. Both groups were treated in a standard way, one group (control) was intraoperatively treated with a placebo solution, and the other (experimental) with a solution containing TXA.

The criteria for inclusion in the study were: age over 18 years and males and females with one-sided inguinal hernia.

The criteria for exclusion from the study were: patients with an inguinoscrotal hernia, patients with a recurrent inguinal hernia after open surgery, patients who did not stop their regular antiplatelet therapy, patients on permanent low-molecular-weight heparin therapy, excluding preoperative one-time prevention, patients with acute disease, exacerbation of chronic disease, patients on dialysis, and patients with hematological diseases excluding anemic syndrome, which meets the conditions for introduction to general endotracheal anesthesia, patients with proven connective tissue disease.

The exclusion criteria also included an intraoperative deviation from standard surgery, which included increased bleeding and excessive tissue trauma, which could be the cause of bleeding that might present a false-positive result for the drug under investigation.

Upon admission to hospital treatment, the patient was assigned two numbers, the first number, which was the serial number, related to the name, surname, and order of patient's surgical procedure, together with the second number, 1 or 2, indicating the recipient of a placebo or drug. The ward nurse, in the operation block and the lead researcher was the only person who was familiar with the numbers and their correlation. Thereupon, the patients underwent standard preoperative preparation, had blood drawn, were operated on, and received standard therapy.

The standard procedure of the Lichtenstein technique was performed for the repair of the inguinal hernia. Each patient was operated on using a uniform method, and the surgery was performed by five experienced general surgeons, each with over five years of specialized experience. For hernioplasty, polypropylene mesh of the same type, characteristics and size 6 × 11 cm or 8 × 15 cm, depending on the size of the inguinal canal, was used for all patients. Prior to inserting, the mesh was shaped accordingly and subsequently fixed along the outer lower edge continuously with a monofilament, non-absorbable suture of thickness 2.0 in the region of the inguinal ligament, from the tubercle to 2–4 cm above the internal inguinal ring. The mesh was further fixed with 3–5 single sutures of the same material along the inner upper edge and with two single sutures on the previously incised mesh to create a new internal inguinal ring. Access to the inguinal canal, preparation and management of the hernial sac, reconstruction of the posterior wall of the inguinal canal, and closure of the inguinal canal were performed in a standardized manner, as a standard procedure in open inguinal hernia repairs.

TXA or placebo was prepared outside the operating room, by the previously authorized person, who did not inform the surgical team about its content. Depending on this, either a medication solution was prepared by drawing two ampoules of TXA, i.e., 10 ml of solution at a concentration of 500 mg / 5 ml, into a 20 ml syringe, followed by adding 10 ml of sterile 0.9% saline solution, or just 20 ml of 0.9% saline solution as a placebo in a syringe of the same specifications.

The professional did not inform anyone about the contents of the syringe. During the operation, the hernia sac or its residues were poured over with the solution, without removal of the residues for at least 1 minute. Further on, 8 ml of solution were poured on the entire site below the aponeurosis of *musculus obliquus externus*, without removal of the residues. Afterwards, an additional 4 ml of solution were poured on the layers above the closed fascia, without removal of the residues for at least 1 minute. Intraoperative, prior to using the solution, hemostasis was achieved by surgical means.

The described methods achieved the effect of a double-blind study.

Postoperatively, the occurrence of hematoma in the operated region was monitored until the postoperative day 7, when most of the bleeding would be visualized [2]. The appearance of a hematoma was considered statistically significant if the width was greater than 2 cm along the entire lower and upper edge of the postoperative wound.

In addition, changes in the blood count were monitored preoperatively and postoperatively, the day before and after the operation. In this way, we analyzed the differences in the blood count in the control and observed groups. We monitored the number of erythrocytes, hemoglobin, and platelets as easily measurable components involved in the coagulation cascade.

On the first postoperative day, patients were examined in detail to record possible complications and hematoma at the site of the operative wound. Also, laboratory analyses were repeated, i.e., blood test results. The wound was then bandaged, and if the treatment was without complications, the patients were discharged for further home and outpatient follow-up.

After discharge, patients were examined after seven days to record clinical signs of bleeding, and other complications.

In the study, among the methods of descriptive statistics, the measure of central tendency, the arithmetic mean, measures of variability and the standard deviation, as well as relative numbers, were used. A simple random sampling system and tables of random numbers were used for randomisation of the patients. Analytical statistics methods were used to identify empirical distributions and methods

Table 1. The age structure of the observed groups

	Tranexamic acid applied							
	No				Yes			
	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum
Age (years)	63.85	11.85	23	85	59.46	13.94	27	87

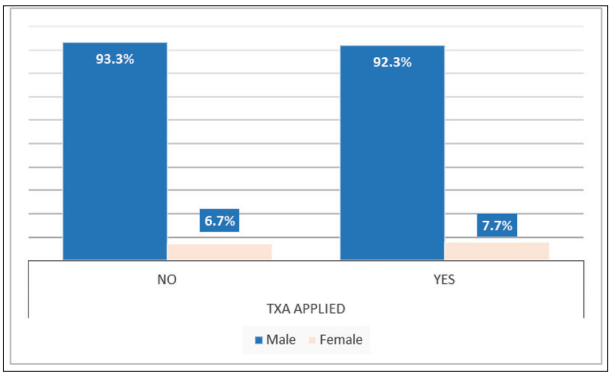

Figure 1. Sex structure of the observed groups

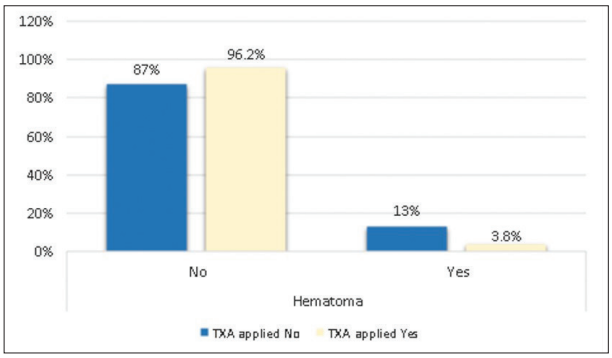

Figure 2. Frequency of occurrence of postoperative hematoma; TXA – tranexamic acid

for assessing the significance of the difference depending on the type of data, the χ^2 test, and t-test for independent samples. The IBM SPSS Statistics (IBM Corp., Armonk, NY, USA) program was used for statistical data processing.

Ethics: Informed consent was obtained from all subjects involved in the study. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Ethics Committee of the Đorđe Joanović Zrenjanin General Hospital (protocol code 01-133/56, date of approval April 10, 2024).

RESULTS

By comparing the demographic characteristics of the patients in both groups, we did not find a statistically significant difference. In the group of patients who received TXA, the average age was 59 years, while in the control group the average age was 64 years. There was no statistically significant difference between the observed groups ($p = 0.076$). The age structure is shown in Table 1.

Figure 1 shows the sex structure of the observed groups, where it was also noted that there was no statistically significant difference ($p = 0.834$). Men were predominant in both groups of patients, 92.3% in the experimental group and 93.3% in the control group.

A statistically significant lower frequency of postoperative hematoma was observed in the group that received TXA during surgery ($p < 0.05$). It was observed that in the experimental group, hematoma appeared in 3.8% of patients, while in the control group, hematoma occurred in 13% of patients. The data are shown in Figure 2.

A statistically significant difference in the number of red blood cells before and after surgery between groups ($p = 0.672$) was not observed. The mean value of red blood cells preoperatively was 4.71×10^{12}/l in the control group, while postoperatively it was 4.58×10^{12}/l. Moreover, in the experimental group, the values were 4.81×10^{12}/l and

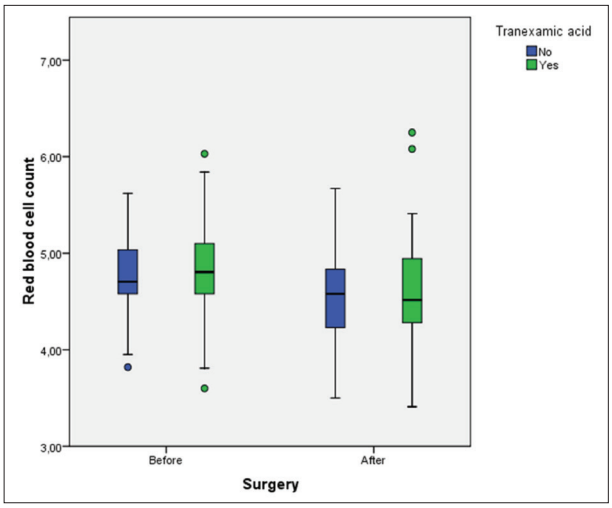

Figure 3. Preoperative and postoperative number of red blood cells in the observed groups

4.52×10^{12}/l preoperatively and postoperatively. The results are shown in Figure 3.

A statistically significant difference was not observed in the mean hemoglobin level preoperatively and postoperatively in the observed groups ($p = 0.924$). The mean value of hemoglobin preoperatively was 145.2 g/l in the control group, while postoperatively it was 137.93 g/l. In the experimental group, the values were 146.23 g/l and 139.31 g/l preoperatively and postoperatively. The values are shown in Figure 4.

The decrease in platelet count was statistically significantly lower in the group of patients who were administered TXA, compared to the group that was given a placebo ($p = 0.030$). The mean value of platelets preoperatively was 235.45×10^9/l in the control group, while postoperatively it was 209.27×10^9/l; in the experimental group, the values were 232.90×10^9/l and 220.06×10^9/l preoperatively and postoperatively. The results are shown in Figure 5.

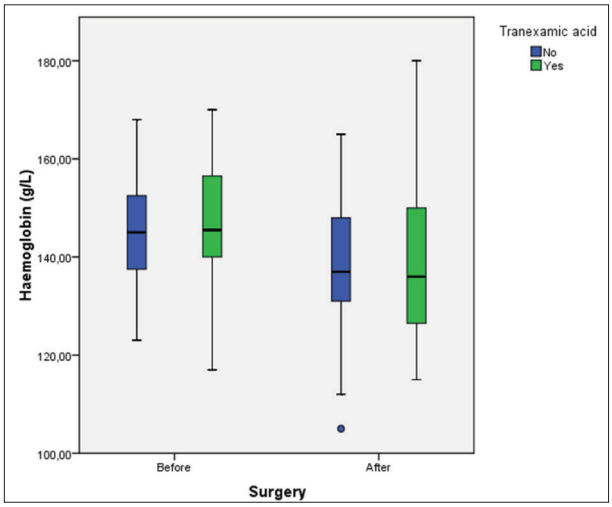

Figure 4. Preoperative and postoperative level of hemoglobin in the observed groups

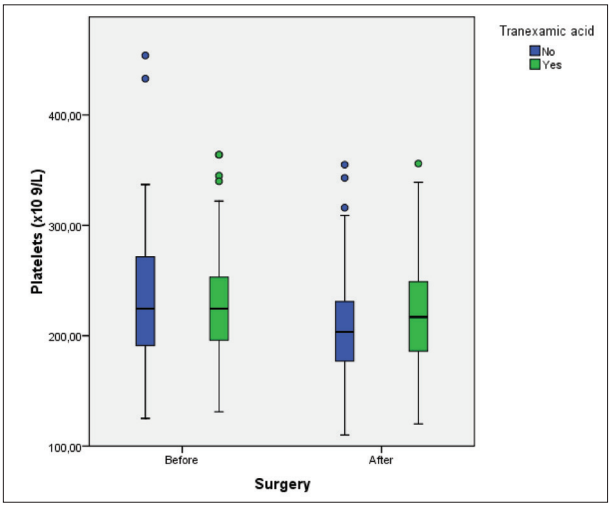

Figure 5. Preoperative and postoperative level of platelets in the observed groups

DISCUSSION

The sex distribution disclosed that men predominated in both the experimental and the control group. The obtained data coincide with the results of other studies, explaining this by the fact that men are exposed to heavier physical efforts through work and physical training. Regarding the age distribution, the results of our study fall roughly between the values of the studies by Agarwal [22] and Burcharth et al. [23].

There is a small number of studies dealing with the use of TXA in abdominal surgery and abdominal wall surgery [16, 24, 25]. The number of subjects in the studies is small, and the possibilities are large, considering the number of operations and potential complications related to postoperative bleeding. Inguinal hernia repair is one of the most common surgeries in the world, and the most common technique for surgery is the Lichtenstein technique. Thus, according to some reports, 275,000 such operations were performed in Germany, while 800,000 such operations were performed in the United States of America in one year [26, 27].

Bleeding is one of the most common complications of this surgery, and it can lead to other complications, such as swelling, hematoma, mesh infection and pain. Hematoma as a sign of bleeding is described with varying frequency, from 1.4–13.6%, depending on the author and the definition of the observed complication [26, 27, 28]. These complications can result in slower patient recovery, additional surgical procedures or reoperations, slower return to regular daily activities and work duties, disability, more expensive treatment, and very rarely death. When this number of complications is multiplied by the number of operations and the percentage of their occurrence, it can be concluded that the prevention of bleeding can have great benefits for the patient and the health system. Hematoma as a sign of postoperative bleeding is the most common complication, which can be easily monitored clinically. Through our study, we determined that the frequency of hematomas in the group that did not receive TXA was 13% (eight patients), according to the given criteria. Of these, one patient had significant clinical bleeding, prolonged hospitalization, monitoring and replacement of blood products, while the frequency of occurrence of hematoma in the group that received TXA was 3.8% (two patients). Comparing these two groups, we found that there is a statistically lower incidence of hematoma in the experimental group. Therefore, it can be concluded that there was less intra- and/or postoperative bleeding in this group. Several other studies have shown similar results [9, 10, 11, 14, 16].

TXA is a potent antifibrinolytic agent, as it blocks the lysine binding site on the plasminogen molecule, thereby preventing its interaction with fibrin. This inhibits the activation of the plasminogen molecule and its conversion to plasmin. This inhibition disrupts the activation of plasminogen and its conversion to plasmin, consequently impeding fibrinolysis and preserving the integrity of the blood clot, thus reducing the risk of bleeding. Moreover, during the application of higher doses of TXA during cardiac surgery, convulsions may occur due to the antagonistic effect on GABA(A) receptors. The primary elimination route is renal, with over 95% of the drug excreted through kidneys. After oral administration, 30–50% of the drug is absorbed and 90% of the drug is excreted from the body within 24 hours when TXA is administered intravenously, while during oral administration 39% of the drug is excreted during the same period. The apparent elimination half-life is close to 2 hours when TXA is administered intravenously, while the mean terminal half-life is approximately 11 hours. Similar pharmacokinetic properties of TXA when administered topically are not available [4, 5].

The mechanism of action of TXA theoretically increases the risk of thromboembolic events, so it is not recommended in patients where this risk is increased [4, 5]. However, in practice, this risk has not been confirmed, as shown in a large number of studies [8, 9, 11]. No increased frequency of vascular side effects such as myocardial infarction, stroke, pulmonary thromboembolism (PTE), or deep vein thrombosis was shown. There was no risk of acute renal failure either. Even a protective effect against such side effects has been shown. However, there was an

increased risk of seizures when high doses of TXA were used in cardiac surgery [8, 29].

The mechanism of action of TXA during local application is the same as during systemic application of TXA, the stabilization of the formed coagulum on severed venous and arterial blood vessels and capillaries. The methods of application are different, by spraying, pouring, local infiltration and covering the surface with gauze and fabric soaked with TXA. Spraying and pouring of tissue (topical application) are most recommended due to surface coverage and ensuring sufficient contact of tissue and TXA. The concentrations used are very different, ranging 1–100 mg/ml, where even the lowest concentration is 100 times higher than the minimum required concentration of TXA in plasma to achieve a beneficial effect, which is 10 μg/ml. So far, no information is available on whether the plasma concentration after topical application of TXA is the same or lower than the tissue concentration. It has not been shown that a greater number of adverse reactions, which we mentioned earlier, occur with topical application of TXA compared to systemic application of TXA or when it is not used at all [9, 10, 11].

We compared the blood count before and after surgery, more precisely the level of erythrocytes and the level of hemoglobin. We wanted to determine whether topical application of TXA affects the level of these two blood count indicators. The results showed that TXA did not affect the level of erythrocytes and hemoglobin postoperatively in the experimental and control groups. From this, it can be concluded that these are not the factors that influence the less frequent occurrence of hematoma in the group where TXA was applied. Kushwaha et al. [12] showed a different result in his study when he measured preoperative and postoperative hemoglobin levels, and when he observed a statistically significant difference in the group where TXA was applied topically compared to the group that received saline solution. Both groups received systemic saline instead of TXA, which was similarly shown in a study by Zhong et al. [13].

On the other hand, the study indicated that the local application of TXA affects the postoperative platelet level, reduces the decline, i.e., consumption, in comparison to the group of patients who were not prescribed TXA, which is contrary to some other studies [13]. It is not clear by which exact mechanism the local application of TXA affects systemic platelet level, however, one possible mechanism is more efficient formation of clot and thrombus on severed blood vessels, thereby reduced consumption of platelets during their formation. Certainly, determining the mechanism of action remains a subject for further research, and this knowledge could be applied in preoperative preparation of all patients, especially for those with lower platelet count.

No adverse reactions during topical application of TXA were recorded, which is consistent with the experience of other authors [9, 10, 11]. The influence of TXA can be indirectly applied to other areas of surgery and invasive procedures. Also, there are still a lot of doubts that need to be resolved, such as, for example, the specific mechanism of action of TXA at the local level, required concentrations, method of application, and length of contact with the tissue.

CONCLUSION

The study determined that the topical application of TXA during inguinal hernia repair using the Lichtenstein technique statistically significantly reduces the occurrence of postoperative hematoma, indicative of postoperative bleeding. Furthermore, the results demonstrated that TXA does not affect the level of erythrocytes and hemoglobin before and after surgery. However, it was proven that the impact of administering TXA led to a lesser drop in the platelet count prior to and post-surgery.

Conflict of interest: None declared.

REFERENCES

1. Weiser TG, Haynes AB, Molina G, Lipsitz SR, Esquivel MM, Uribe-Leitz T, et al. Estimate of the global volume of surgery in 2012: an assessment supporting improved health outcomes. Lancet. 2015;385 Suppl 2:S11. [DOI: 10.1016/S0140-6736(15)60806-6] [PMID: 26313057]
2. Halme ALE, Roshanov PS, Tornberg SV, Lavikainen LI, Devereaux PJ, Tikkinen KAO. Timing of major postoperative bleeding among patients undergoing surgery. JAMA Netw Open. 2024;7(4):e244581. [DOI: 10.1001/jamanetworkopen.2024.4581] [PMID: 38564217]
3. Roshanov PS, Eikelboom JW, Sessler DI, Kearon C, Guyatt GH, Crowther M, et al. Bleeding independently associated with mortality after non-cardiac surgery (BIMS): an international prospective cohort study establishing diagnostic criteria and prognostic importance. Br J Anaesth. 2021;126(1):163–71. [DOI: 10.1016/j.bja.2020.06.051] [PMID: 32768179]
4. DrugBank Online [homepage on the Internet]. OMx Personal Health Analytics Inc.; 2024 [updated 2024; cited 2024]. Available from: https://go.drugbank.com/drugs/DB00302
5. Mediately [homepage on the Internet]. Ljubljana: Mediately; 2023 [updated 2023; cited 2024]. Available from: https://mediately.co/rs/drugs/GJXwwATqe6FSDsap2SoZZ8gmWfY/tranexamic-medochemie-500mg-5ml-rastvor-za-injekciju
6. Heyns M, Knight P, Steve AK, Yeung JK. A single preoperative dose of tranexamic acid reduces perioperative blood loss: a meta-analysis. Ann Surg. 2021;273(1):75–81. [DOI: 10.1097/SLA.0000000000003793] [PMID: 32224739]
7. Brown NJ, Choi EH, Gendreau JL, Ong V, Himstead A, Lien BV, et al. Association of tranexamic acid with decreased blood loss in patients undergoing laminectomy and fusion with posterior instrumentation: a systematic review and meta-analysis. J Neurosurg Spine. 2022;36(4):686–93. [DOI: 10.3171/2021.7.SPINE202217] [PMID: 34740174]
8. Hong P, Liu R, Rai S, Liu J, Ding Y, Li J. Does tranexamic acid reduce the blood loss in various surgeries? An umbrella review of state-of-the-art meta-analysis. Front Pharmacol. 2022;13:887386. [DOI: 10.3389/fphar.2022.887386] [PMID: 35662737]
9. Teoh WY, Tan TG, Ng KT, Ong KX, Chan XL, Tsan SEH, et al. Prophylactic topical tranexamic acid versus placebo in surgical patients: a systematic review and meta-analysis. Ann Surg. 2021;273(4):676–83. [DOI: 10.1097/SLA.0000000000003896] [PMID: 32282377]
10. Ausen K, Fossmark R, Spigset O, Pleym H. Safety and efficacy of local tranexamic acid for the prevention of surgical bleeding in soft-tissue surgery: a review of the literature and recommendations for plastic surgery. Plast Reconstr Surg. 2022;149(3):774–87. [DOI: 10.1097/PRS.0000000000008884] [PMID: 35196701]

11. Ker K, Beecher D, Roberts I. Topical application of tranexamic acid for the reduction of bleeding. Cochrane Database Syst Rev. 2013;7:CD010562. [DOI: 10.1002/14651858.CD010562.pub2] [PMID: 23881695]
12. Kushwaha NS, Singh S, Kumar S, Singh A, Abbas MB, Deshwal S, et al. Local versus systemic tranexamic acid in total hip arthroplasty in young adults. Cureus. 2023;15(3):e36230. [DOI: 10.7759/cureus.36230] [PMID: 37069867]
13. Zhong L, Liu Y, Wang Y, Wang H. Effects of local administration of tranexamic acid on reducing postoperative blood loss in surgeries for closed Sanders III–IV calcaneal fractures: a randomized controlled study. Indian J Orthop. 2021;55(2):418–25. [DOI: 10.1007/s43465-021-00417-2] [PMID: 34306556]
14. Kang H, Hwang SH. Does topical application of tranexamic acid reduce intra-operative bleeding in sinus surgery during general anesthesia? Braz J Otorhinolaryngol. 2020;86(1):111–8. [DOI: 10.1016/j.bjorl.2019.08.006] [PMID: 31653606]
15. Fuzi J, Budiono GR, Meller C, Jacobson I. Tranexamic acid in otorhinolaryngology: a contemporary review. World J Otorhinolaryngol Head Neck Surg. 2020;7(4):328–37. [DOI: 10.1016/j.wjorl.2020.05.010] [PMID: 34632348]
16. Ghaffari Hamedani SM, Akbari A, Sayaydi S, Zakariaei Z, Moosazadeh M, Boskabadi J, et al. Topical application of tranexamic acid to control bleeding in inguinal hernia surgery candidate patients: a randomized controlled trial. Ann Med Surg. 2021;69:102683. [DOI: 10.1016/j.amsu.2021.102683] [PMID: 34429952]
17. Eikebrokk TA, Vassmyr BS, Ausen K, Gravastrand C, Spigset O, Pukstad B. Cytotoxicity and effect on wound re-epithelialization after topical administration of tranexamic acid. BJS Open. 2019;3(6):840–51. [DOI: 10.1002/bjs5.50192] [PMID: 31832591]
18. Ausen K, Hagen AI, Østbyhaug HS, Olafsson S, Kvalsund BJ, Spigset O, et al. Topical moistening of mastectomy wounds with diluted tranexamic acid to reduce bleeding: randomized clinical trial. BJS Open. 2020;4(2):216–24. [DOI: 10.1002/bjs5.50248] [PMID: 32207575]
19. Çirakli A, Gürgör PN, Uzun E, Erdem H, Çankaya S, Baş O. Local application of tranexamic acid affects tendon healing negatively in the late period. Eklem Hastalik Cerrahisi. 2018;29(1):20–6. [DOI: 10.5606/ehc.2018.56675] [PMID: 29526155]
20. Çevik HB, Eceviz E, Çilingir Kaya ÖT, Ercan F, Çeçen GS. The effect of topical and systemic tranexamic acid on fracture healing in rats. Acta Orthop Traumatol Turc. 2020;54(2):207–12. [DOI: 10.5152/j.aott.2020.02.44] [PMID: 32254038]
21. Degirmenci E, Ozturan KE, Sahin AA, Yilmaz F, Kaya YE. Effects of tranexamic acid on the recovery of osteochondral defects treated by microfracture and acellular matrix scaffold: an experimental study. J Orthop Surg Res. 2019;14(1):105. [DOI: 10.1186/s13018-019-1144-7] [PMID: 30992060]
22. Agarwal PK. Study of demographics, clinical profile and risk factors of inguinal hernia: a public health problem in elderly males. Cureus. 2023;15(4):e38053. [DOI: 10.7759/cureus.38053] [PMID: 37122980]
23. Burcharth J, Pedersen M, Bisgaard T, Pedersen C, Rosenberg J. Nationwide prevalence of groin hernia repair. PLoS One. 2013;8(1):e54367. [DOI: 10.1371/journal.pone.0054367] [PMID: 23342139]
24. Zubair R, Mirza MR, Habib L, Iftikhar J, Zehra B. Role of tranexamic acid in prevention of seroma formation after ventral hernioplasty. Pak J Surg. 2020;36(2):126–9.
25. Kovalev V, Dong F, Bagheri S, Wong D, Wi M. Effectiveness of tranexamic acid in reducing hemorrhage in isolated blunt solid organ injury. Cureus. 2021;13(12):e20473. [DOI: 10.7759/cureus.20473] [PMID: 35070532]
26. Hajili K, Hernandez AV, Otten J, Richards D, Rudroff C. Risk factors for early and late morbidity in patients with cardiovascular disease undergoing inguinal hernia repair with a tailored approach: a single-center cohort study. BMC Surg. 2023;23(1):11. [DOI: 10.1186/s12893-023-01905-y] [PMID: 36641449]
27. Zeb MH, Pandian TK, Khatib MM, Naik ND, Chandra A, Morris DS, et al. Risk factors for postoperative hematoma after inguinal hernia repair: an update. J Surg Res. 2016;205(1):33–7. [DOI: 10.1016/j.jss.2016.06.002] [PMID: 27620996]
28. Neumayer L, Giobbie-Hurder A, Jonasson O, Fitzgibbons R, Dunlop D, Gibbs J. Open mesh versus laparoscopic mesh repair of inguinal hernia. N Engl J Med. 2004;350(18):1819–27. [DOI: 10.1056/NEJMoa040093] [PMID: 15107485]
29. Guo J, Gao X, Ma Y, Lv H, Hu W, Zhang S, et al. Different dose regimens and administration methods of tranexamic acid in cardiac surgery: a meta-analysis of randomized trials. BMC Anesthesiol. 2019;19(1):129. [DOI: 10.1186/s12871-019-0772-0] [PMID: 31307381]

Ефекат локалне примене транексаминске киселине на појаву постоперативног хематома после операције ингвиналне хернije Лихтенштајновом техником

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САЖЕТАК

Увод/Циљ Циљ студије је био да се утврди да ли локална примена транексаминске киселине (*TXA*) посипањем хируршке ране смањује учесталост појаве постоперативног хематома, тј. постоперативног крварења у рани након операције ингвиналне хернije Лихтенштајновом техником.

Методе Спровели смо проспективну, рандомизирану, дупло слепу клиничку студију. Сто двадесет болесника било је подељено у две групе; једној групи је апликована *TXA* током операције, а другој плацебо. Даље, групе су поређене и анализиране према полу, старосној доби, учесталости постоперативног крварења и преоперативном и постоперативном нивоу еритроцита, хемоглобина и тромбоцита.

Резултати Резултати указују да се постоперативни хематом појављивао статистички значајно ређе у групи којој је ординирана *TXA* у односу на групу којој је ординиран плацебо ($p < 0,05$). Учесталост појављивања постоперативног хематома у групи која је третирана са *TXA* била је 3,8%, док је учесталост у другој групи била 13%. Постојала је статистички значајна разлика у нивоу тромбоцита пре и после операције међу поређеним групама.

Закључак Утврдили смо да локална примена *TXA* смањује појаву постоперативног хематома и самим тим постоперативног крварења, као и постоперативни пад тромбоцита.

Кључне речи: операција ингвиналне хернije; транексаминска киселина; хематом; хемоглобин; еритроцити; тромбоцити

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Evaluation of the prognostic performance of the Rockall and Glasgow-Blatchford scoring systems in non-variceal upper gastrointestinal bleeding

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SUMMARY

Introduction/Objective Upper gastrointestinal bleeding is a significant medical emergency requiring prompt assessment and intervention. Various risk stratification tools, including the Rockall Score and Glasgow-Blatchford Score (GBS), are used to predict clinical outcomes such as mortality, intensive care unit admission, and the need for blood transfusion.

Methods This study analyzed a cohort of 199 patients admitted to our hospital for non-variceal upper gastrointestinal bleeding between October 1, 2020, and October 1, 2024. Demographic data, vital signs (pulse rate, systolic and diastolic blood pressure), length of hospital and ICU stay, comorbidities, and medication use were recorded. The Rockall Score and GBS were calculated for each patient, and their predictive accuracy was assessed using sensitivity and specificity analyses.

Results The GBS (AUC = 0.887) demonstrated superior predictive performance for blood transfusion compared to the Rockall Score (AUC = 0.786, $p < 0.001$). However, both scores exhibited poor predictive ability for ICU admission (AUC = 0.624 *vs*. 0.605, respectively, $p < 0.05$), with Rockall outperforming GBS. For mortality prediction, both scores performed similarly (Rockall: AUC = 0.847, GBS: AUC = 0.837, $p = 0.239$), indicating no significant difference.

Conclusion GBS outperforms the Rockall Score in predicting blood transfusion need, while both scores show poor ICU admission prediction, with Rockall performing slightly better. For mortality prediction, both scores are comparable. GBS is preferable for transfusion assessment, but additional factors may improve ICU and mortality predictions.

Keywords: gastrointestinal bleeding; Rockall Score; Glasgow-Blatchford Score; mortality; transfusion

INTRODUCTION

Upper gastrointestinal (GI) bleeding represents a serious medical emergency that can pose significant risks to a patient's life. This type of bleeding, originating from the upper part of the digestive system – which includes the esophagus, stomach, and the first part of the small intestine – demands careful and diligent monitoring due to its potential to lead to severe complications such as shock and even death [1]. The nature of upper GI bleeding can vary, ranging from minor oozing to massive hemorrhage, and can occur due to a variety of underlying conditions, including ulcers, varices, or malignancies. One of the most concerning aspects is the risk of recurrent bleeding, which may exacerbate the patient's condition and necessitate further medical interventions. In such cases, urgent upper endoscopy within 24 hours is recommended as the cornerstone of both diagnosis and therapeutic intervention, allowing for timely identification and control of the bleeding source. Early endoscopy has been shown to reduce transfusion requirements, length of hospital stay, and mortality, particularly in high-risk patients [2]. Therefore, a well-structured and proactive approach to managing upper GI bleeding is crucial. This involves rapid assessment, stabilization of vital signs, and identification of the bleeding source. Effective management not only focuses on immediate treatment but also emphasizes the need for ongoing surveillance and follow-up care to reduce the risk of recurrence and improve patient outcomes. Understanding these complexities is essential for healthcare providers in delivering safe and effective care to affected individuals [3].

To effectively categorize GI bleeding incidents and assess the associated rates of rebleeding and mortality, various risk assessment tools have been developed. Among these, two of the most prominent tools are the Glasgow-Blatchford Score (GBS) and the Rockall Score [4]. The Rockall Score is a comprehensive risk assessment tool that combines both pre-endoscopy and post-endoscopy factors to more accurately evaluate a patient's risk. It considers clinical variables such as age, comorbidities, and the severity of the bleeding observed during endoscopy, allowing healthcare providers to stratify patients based on their likelihood of rebleeding or death resulting from the bleeding event [5]. In contrast, the GBS focuses exclusively on pre-endoscopy variables to gauge the initial severity of the bleeding. This score

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takes into account symptoms presented by the patient, vital signs, and any underlying medical conditions (comorbidities). It is particularly valuable in emergency settings because it helps clinicians quickly identify patients who may require urgent intervention based on their initial presentation. Both scoring systems are essential in guiding clinical decision-making, risk stratification, and treatment approaches for patients experiencing GI hemorrhages [6].

The primary aim of this study was to conduct a comprehensive comparison between the Rockall and Glasgow-Blatchford scoring systems, both of which have been developed to assess patients presenting with GI bleeding. Specifically, the study aimed to evaluate the effectiveness of these scoring systems in predicting three critical clinical outcomes: the need for blood transfusions, the likelihood of requiring admission to an ICU, and the overall mortality associated with GI bleeding. By analyzing these outcomes, the study sought to determine which scoring system provides better clinical guidance for clinicians in managing patients with GI hemorrhages.

METHODS

This study examined a cohort of 199 patients who were admitted to Gazi Yaşargil Training and Research Hospital for treatment of upper gastrointestinal bleeding over a period spanning from October 1, 2020, to October 1, 2024. We meticulously gathered demographic information regarding each patient, including their age and gender at the time of admission. Additionally, we recorded vital signs, such as pulse rate, systolic and diastolic blood pressure, which are critical indicators of a patient's cardiovascular status. We also tracked the duration of each patient's stay in the intensive care unit, as well as their overall hospital admission length. Furthermore, we documented any existing comorbidities of the patients to provide a comprehensive overview of their health profiles. This study was conducted in accordance with the Declaration of Helsinki.

Patients were enrolled in the study according to predefined inclusion criteria outlined below:

Inclusion criteria:

- (≥ 18 years) with confirmed upper gastrointestinal (GI) bleeding based on endoscopic findings.

Exclusion criteria:

- No endoscopy performed or no endoscopic evidence of upper GI bleeding
- Upper GI bleeding due to varices or malignancy
- Referral to other medical centers
- Age < 18 years
- Incomplete or missing medical records.

The Rockall Score and GBS of the patients were recorded, and analyses of their sensitivity and specificity were performed.

Calculation of the Rockall Score

The Rockall Score is a clinical scoring system used to assess the risk of mortality and rebleeding in patients with upper gastrointestinal bleeding. It consists of pre-endoscopic and post-endoscopic components.

1. Pre-endoscopic Rockall Score

The initial (pre-endoscopic) Rockall score is calculated based on the three parameters shown in Table 1.

2. Complete Rockall Score (post-endoscopic)

Once endoscopy is performed, two additional parameters are included to refine the risk assessment (Table 2).

Table 1. Pre-Endoscopic Rockall Scoring System

Category	Criteria	Score
Age	< 60 years	0
	60–79 years	1
	≥ 80 years	2
Shock (hemodynamic status)	No shock (SBP ≥ 100 mmHg and HR < 100 bpm)	0
	Tachycardia (HR ≥ 100 bpm) but SBP ≥ 100 mmHg	1
	Hypotension (SBP < 100 mmHg)	2
Comorbidities	No major comorbidity	0
	Cardiac failure, ischemic heart disease, chronic kidney disease, malignancy, or other significant comorbidities	2
	Metastatic malignancy	3

SBP – systolic blood pressure; HR – heart rate; BPM – beats per minute; maximum pre-endoscopic score: 7

Table 2. Complete Rockall Scoring System (post endoscopic)

Category	Criteria	Score
Endoscopic diagnosis	No lesion, Mallory–Weiss tear	0
	All other diagnoses	1
	Malignancy of upper GI tract	2
Signs of recent hemorrhage	No stigmata of recent hemorrhage	0
	Blood in upper GI tract or adherent clot	2
	Active bleeding (spurting or oozing)	2

Maximum complete Rockall Score: 11

Interpretation of the Rockall Score

- Low Risk (0–2 points): Low mortality and rebleeding risk; may be managed with early discharge.
- Moderate Risk (3–4 points): Increased risk; requires closer monitoring.
- High Risk (≥ 5 points): High mortality and rebleeding risk; often requires ICU admission and intensive management [1].

Calculation of GBS

GBS is calculated using multiple clinical and laboratory parameters. The blood urea nitrogen (BUN) level contributes to the score, with values below 6.5 mmol/L receiving 0 points, 6.5–8 mmol/L scoring 2 points, 8–10 mmol/L scoring 3 points, 10–25 mmol/L scoring 4 points, and above 25.0 mmol/L scoring 6 points. Hemoglobin levels are also considered separately for men and women. In men, ≥ 13 g/dL is scored as 0, 12–12.9 g/dL as 1, 10–11.9 g/dL as 3, and < 10 g/dL as 6. In women, ≥ 12 g/dL is scored as 0, 10–11.9 g/dL as 1, and < 10 g/dL as 6 (Table 3).

Systolic blood pressure (SBP) is another important factor, where values ≥ 110 mmHg receive 0 points,

100–109 mmHg receive 1 point, 90–99 mmHg receive 2 points, and < 90 mmHg receive 3 points. A pulse rate of ≥ 100 bpm contributes 1 point to the total score. The presence of melena (black stools) is given 1 point, while syncope adds 2 points. If the patient has hepatic disease, an additional 2 points are assigned. Similarly, the presence of cardiac failure also contributes 2 points to the overall score (Table 3).

Table 3. Glasgow-Blatchford Scoring System

Parameter	Criteria	Score
Blood urea nitrogen (mmol/L)	< 6.5	0
	6.5–8	2
	8–10	3
	10–25	4
	> 25	6
Hemoglobin (g/dL, Men)	≥ 13	0
	12–12.9	1
	10–11.9	3
	< 10	6
Hemoglobin (g/dL, Women)	≥ 12	0
	10–11.9	1
	< 10	6
Systolic blood pressure (mmHg)	≥ 110	0
	100–109	1
	90–99	2
	< 90	3
Pulse rate (bpm)	≥ 100	1
Melena	Present	1
Syncope	Present	2
Hepatic disease	Present	2
Cardiac failure	Present	2

BPM – beats per minute

Interpretation of GBS

- Score = 0 → Very low risk; outpatient management is safe.
- Score ≥ 1 → Increased risk; hospitalization and further evaluation are recommended.
- Score ≥ 6 → High risk of severe bleeding and mortality; requires urgent intervention (endoscopy, transfusion, ICU admission) [7].

Statistics

The Kolmogorov–Smirnov test was employed to determine whether the data followed a normal distribution. For data that were normally distributed, results are presented as mean ± SD, while data that were not normally distributed are shown as median (IQR). If the non-categorical data were normally distributed, comparisons were made using the Student's t-test. For data that were not normally distributed, the Mann–Whitney U test was used for comparison. Categorical data were analyzed using the χ^2 test. Sensitivity and specificity were assessed using the ROC curve, and results were compared with the DeLong test. A p-value of less than 0.05 was considered statistically significant. The program used for statistical analysis was IBM SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA).

Ethics: Ethics committee approval for this study was obtained from the Ethics Committee of Gazi Yaşargil Education and Research Hospital on March 28, 2024, with approval number 403. The study was conducted in accordance with the Declaration of Helsinki and its ethical principles.

RESULTS

ICU admission and sex distribution: Among male patients, 110 individuals (74.8%) were admitted to the ICU, while 37 individuals (25.2%) were not. Among female patients, 38 individuals (73.1%) were admitted to the ICU, whereas 14 individuals (26.9%) were not. A total of 199 patients were evaluated for ICU admission, with a p-value of 0.803; this indicates no statistically significant difference (p = 0.803) (Table 4).

Mortality and sex distribution: Among male patients, 138 individuals (93.2%) were discharged, while nine individuals (6.8%) died. Among female patients, 48 individuals (92.3%) were discharged, whereas four individuals (7.7%) died. A total of 199 patients were assessed for survival status, with a p-value of 0.694. There is no significant difference in mortality rates between male and female patients (p = 0.694) (Table 5).

Table 4. Admission to the intensive care unit (ICU) according to sex

Sex	Admitted to ICU	Not admitted to ICU	Total
Male	110	37	147
Female	38	14	52
Total	148	51	199

Table 5. Mortality according to sex

Sex	Discharged	Exitus (death)	Total
Male	138	9	147
Female	48	4	52
Total	186	13	199

p-value: 0.694

Age distribution: The median age of male patients was 57 years (IQR 72), while the median age of female patients was 77.5 years (IQR 80), and this age difference was found to be statistically significant (p < 0.001), suggesting that female patients with upper GI bleeding were generally older than their male counterparts (Table 7).

Vital signs: The median pulse rate was 88 bpm for both males (IQR 81) and females (IQR 85), with no statistically significant difference between the groups (p = 0.645) (Table 5). Similarly, the median systolic blood pressure was 115 mmHg in males (IQR 135) and 110.5 mmHg in females (IQR 130), showing no significant difference (p = 0.746) (Table 6). Although the median diastolic blood pressure was slightly higher in males (70 mmHg, IQR 65) compared to females (63.5 mmHg, IQR 68), the difference approached but did not reach statistical significance (p = 0.063) (Table 7).

Length of stay: The total length of hospital stay was similar between male and female patients, with a median

of five days (IQR 150) for males and 5.5 days (IQR 33) for females, showing no statistically significant difference (p = 0.835) (Table 6). Likewise, the median length of stay in the ICU was two days for both sexes – males (IQR 151) and females (IQR 35) – with no significant difference observed (p = 0.608) (Table 7).

Endoscopic results: Among 199 patients who underwent endoscopy, peptic ulcer was the most common finding, observed in 91% of cases. Other lesions were infrequent, including erosive gastritis and esophagitis (2% each), angiodysplasia (1.5%), and erosive bulbitis (1%). Rare findings (0.5% each) included bulbar diverticulum, gastric antral vascular ectasia, esophageal ulcer, and duplicate entries of erosive gastritis and bulbitis (Table 6).

Table 6. Endoscopic results of the patients

Endoscopic lesion	Frequency	Percentage
Peptic ulcer	181	91%
Erosive gastritis	4	2%
Esophagitis	4	2%
Angiodysplasia	3	1.5%
Erosive bulbitis	2	1%
Bulbar diverticulum	1	0.5%
Erosive bulbitis *(alternate entry)*	1	0.5%
Erosive gastritis *(alternate entry)*	1	0.5%
Gastric antral vascular ectasia	1	0.5%
Esophageal ulcer	1	0.5%

Clinical scores: The GBS had a median value of 11 (IQR 16) for males and 11.5 (IQR 16) for females, with a statistically significant difference observed (p = 0.012), indicating a slightly higher risk profile in female patients (Table 6). Similarly, the Rockall Score was significantly higher in females, with a median of 5 (IQR 8) compared to 3 (IQR 8) in males (p = 0.01), suggesting a greater likelihood of adverse outcomes among female patients (Table 7).

Laboratory and transfusion parameters: The mean hemoglobin level was significantly lower in female patients (7.63 ± 2.16 g/dL) compared to males (9.72 ± 2.92 g/dL), with a p-value of < 0.001, indicating a highly significant sex difference (Table 6). However, the median number of erythrocyte transfusions was the same for both sexes at two units, though the IQR was 33 for males and 22 for females; this difference was not statistically significant (p = 0.11) (Table 7).

Table 7. Descriptive analysis of patients according to sex

Parameter	Male (n = 147)	Female (n = 52)	p
Age (years) median ± IQR	57 (18–90)	77.5 (20–100)	**< 0.001**
Pulse (beats/minute) median ± IQR	88 (55–136)	88 (60–145)	0.645
SBP (mmHg) median ± IQR	115 (65–200)	110.5 (75–205)	0.746
DBP (mmHg) median ± IQR	70 (35–100)	63.5 (42–110)	0.063
LOS (days) median ± IQR	5 (1–151)	5.5 (2–35)	0.835
LOS in ICU (days) median ± IQR	2 (0–151)	2 (0–35)	0.608
Glasgow-Blatchford Score median ± IQR	11 (2–18)	11.5 (1–17)	**0.012**
Rockall Score median ± IQR	3 (1–9)	5 (1–9)	**0.01**
Hemoglobin mean ± SD	9.72 ± 2.92	7.63 ± 2.16	**< 0.001**
Number of transfusions (erythrocyte) median ± IQR	2 (0–33)	2 (0–22)	0.11

SBP – systolic blood pressure; DBP – diastolic blood pressure; LOS – length of stay; ICU – intensive care unit; IQR – interquartile range;
p-values < 0.05 were considered statistically significant

1. Blood transfusion prediction
- GBS: AUC = 0.887 (95% CI: 0.835–0.932)
- Rockall: AUC = 0.786 (95% CI: 0.717–0.844)
- Z = 35.16, p < 0.001 → GBS significantly outperforms Rockall (Figure 1).

2. ICU admission prediction
- Rockall: AUC = 0.624 (95% CI: 0.531–0.714)
- GBS: AUC = 0.604 (95% CI: 0.512–0.682)
- Z = -7.87, p < 0.05 → Rockall slightly better, but both scores show poor to fair predictive value (Figure 2).

3. Mortality Prediction
- Rockall: AUC = 0.847 (95% CI: 0.735–0.937)
- GBS: AUC = 0.837 (95% CI: 0.704–0.939)
- Z = -1.18, p = 0.239 → No significant difference; both show good predictive performance (Figure 3).

DISCUSSION

This study included 199 patients who presented with upper GI bleeding and were being followed up at our hospital. Demographic data of all patients were recorded. The primary objective of the study was to evaluate the role of the Rockall and GB scoring systems – which are specifically developed for patients with GI bleeding –in predicting the need for blood transfusion and ICU admission. Additionally, the study aimed to assess the effectiveness of these scoring systems in predicting mortality.

In upper GI bleeding, there is a general male predominance, with the condition typically occurring at a ratio of approximately 2:1 in favor of males. Additionally, male patients tend to be younger at the time of diagnosis compared to female patients [8, 9]. In our study, the male-to-female ratio was consistent with these previously reported trends, with 147 male patients and 52 female patients included in the analysis. When comparing age distribution, male patients had a median age of 57 years (IQR 72), whereas female patients had a median age of 77.5 years (IQR 80). This indicates that female patients were significantly older than their male counterparts, and this difference was found to be statistically significant (p < 0.001). Although significant differences were observed between the two groups in terms of numerical distribution and age, statistical analyses revealed no significant association between gender and ICU admission rates (p = 0.803) or gender and mortality rates (p = 0.694). This suggests that while demographic characteristics differ, gender does not appear to be an independent predictor of ICU requirement or mortality risk in patients with upper GI bleeding.

In the comparison of vital signs and laboratory parameters between the two groups, no statistically significant differences were generally observed. This included pulse

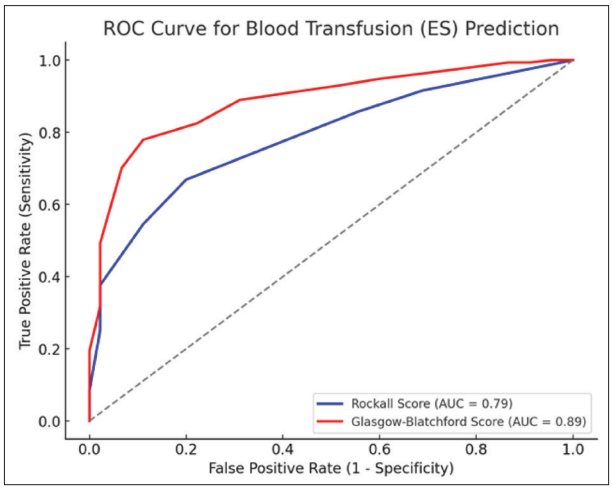

Figure 1. Receiver operating characteristic (ROC) curve of Rockall and Glasgow-Blatchford Scores for transfusion need

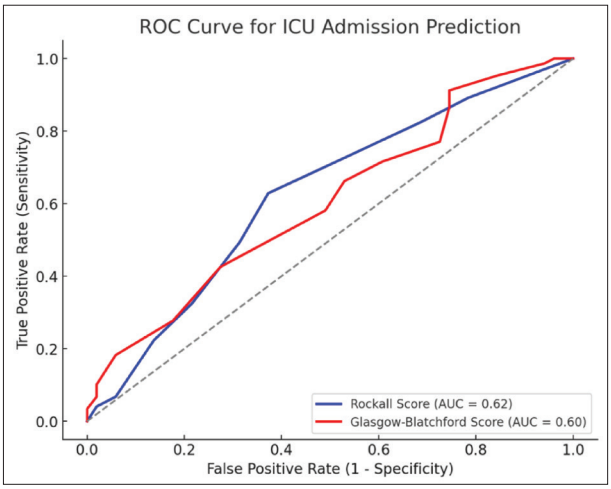

Figure 2. Receiver operating characteristic (ROC) curve of Rockall and Glasgow-Blatchford Scores for ICU admission prediction

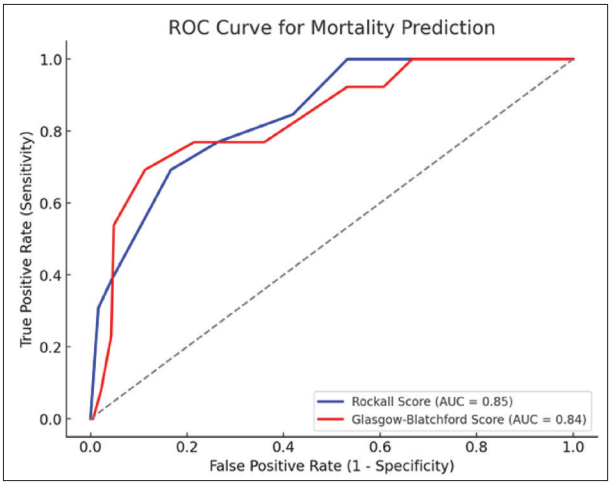

Figure 3. Receiver operating characteristic (ROC) curve of Rockall and Glasgow-Blatchford Score for mortality

rate, systolic blood pressure, and diastolic blood pressure, with p-values of 0.645, 0.746, and 0.063, respectively. Similarly, no significant differences were detected in terms of ICU admission rates ($p = 0.835$) or overall hospital length of stay ($p = 0.608$). However, a statistically significant difference was found in the hemoglobin levels at the time of hospital admission ($p < 0.001$). This difference may be attributed to the lower hemoglobin levels observed in female patients compared to males, which could be associated with the older age profile of female patients in this study. Studies suggest that lower baseline hemoglobin levels in women are often linked to physiological factors, such as menstrual blood loss and differences in iron storage capacity, as well as age-related declines in hematopoietic function [10]. Furthermore, older patients – especially postmenopausal women – may have reduced erythropoietin production and lower bone marrow responsiveness, contributing to their increased susceptibility to anemia [11]. In addition, the lower hemoglobin levels observed in female patients compared to their male counterparts may be influenced by multiple factors, such as chronic nutritional deficiencies (e.g., iron deficiency) and the frequent use of non-steroidal anti-inflammatory drugs, which are known to cause gastrointestinal mucosal damage and bleeding. These factors, individually or in combination, may contribute to the higher prevalence of anemia in women [12].

Gastrointestinal bleeding poses a significant risk in terms of hospital admissions and mortality. To minimize this risk and improve patient management, various risk scoring systems have been developed to assess disease severity, predict clinical outcomes, and guide treatment decisions effectively [13]. This condition has multiple clinical and economic implications, particularly affecting gastrointestinal interventions and healthcare resource utilization. Therefore, it is crucial for clinicians to be aware of these potential outcomes to optimize patient management and decision-making [14]. Among the risk scoring systems developed for this purpose, the Rockall Score and the GBS are among the most widely used. These scoring systems provide valuable insights into the need for blood transfusion, ICU admission, and overall mortality risk, helping clinicians make informed decisions in the management of gastrointestinal bleeding [15]. In the study conducted by Robertson et al. [16], the GBS was found to be superior to the Rockall Score in predicting the need for blood transfusion. However, in terms of mortality prediction, both scoring systems demonstrated comparable accuracy [16].

In our study, the GBS demonstrated superior performance in predicting the need for blood transfusion compared to the Rockall Score, and this difference was found to be statistically significant (AUC = 0.887 *vs.* 0.786, Z-score = 35.16, $p < 0.001$). This finding highlights the practical advantage of using GBS in transfusion assessment, as it offers a more effective and convenient tool for clinical decision-making. However, when evaluating ICU admission, both the Rockall and GBS scores had relatively low AUC values, indicating poor predictive power (AUC = 0.624 *vs.* 0.605). Despite the statistically significant

difference ($p < 0.05$), the low AUC values suggest that neither scoring system is highly reliable for predicting ICU admission. Nevertheless, the Rockall Score outperformed GBS in this context. Regarding mortality prediction, the Rockall Score (AUC = 0.847) and GBS (AUC = 0.837) exhibited similar performance, with no statistically significant difference between the two scores ($p = 0.239$). This suggests that both scoring systems have comparable predictive power in estimating mortality risk in patients with upper GI bleeding.

CONCLUSION

GBS is superior to the Rockall Score in predicting blood transfusion need, making it a more practical tool for clinical decision-making. However, both scores show poor predictive power for ICU admission, with the Rockall Score performing slightly better. In mortality prediction, both scores are comparable with no significant difference. While GBS is preferable for transfusion assessment, additional factors may be needed to improve ICU and mortality predictions.

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Contributions: JK: conceptualization, formal analysis, methodology, writing – original draft; ÖFA: data collection, writing; MO: writing – review and editing; IS: conceptualization, editing.

Availability of data and materials: The authors confirm that most of the data used in this article can be found in Tables 1–6, Figures 1, 2 and 3. Any additional data are available on request.

Conflict of interest: None declared.

REFERENCES

1. Antunes C, Tian C, Copelin EL II. Upper gastrointestinal bleeding. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025–. [PMID: 29262121]
2. DiGregorio AM, Alvey H. Gastrointestinal bleeding. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025–. [PMID: 30725976]
3. Laine L, Barkun AN, Saltzman JR, Martel M, Leontiadis GI. ACG clinical guideline: upper gastrointestinal and ulcer bleeding. Am J Gastroenterol. 2021;116(5):899–917. [DOI: 10.14309/ajg.0000000000001245] [PMID: 33929377]
4. Taşlıdere B, Keskin EB, Özdemir S, Atsız A, Sönmez E. Comparison of Glasgow-Blatchford and new risk scores to predict outcomes in patients with acute upper GI bleeding. Bezmialem Sci. 2023;11(1):100–7. [DOI: 10.14235/bas.galenos.2022.80299]
5. Monteiro S, Gonçalves TC, Magalhães J, Cotter J. Upper gastrointestinal bleeding risk scores: who, when and why? World J Gastrointest Pathophysiol. 2016;7(1):86–96. [DOI: 10.4291/wjgp.v7.i1.86] [PMID: 26909231]
6. Arıkoğlu S, Tezel O, Büyükturan G, Başgöz BB. The efficacy and comparison of upper gastrointestinal bleeding risk scoring systems on predicting clinical outcomes among emergency-unit patients. BMC Gastroenterol. 2025;25(1):93. [DOI: 10.1186/s12876-025-03684-7] [PMID: 39972434]
7. Blatchford O, Murray WR, Blatchford M. A risk score to predict need for treatment for upper-gastrointestinal haemorrhage. Lancet. 2000;356(9238):1318–21. [DOI: 10.1016/S0140-6736(00)02816-6] [PMID: 11073021]
8. Orpen-Palmer J, Stanley AJ. A review of risk scores within upper gastrointestinal bleeding. J Clin Med. 2023;12(11):3678. [DOI: 10.3390/jcm12113678] [PMID: 37297873]
9. Sunil T, George A, Sankar G, Jagan G. A study on upper gastrointestinal endoscopic findings in patients admitted with upper GI bleed. J Res Med Dent Sci. 2021;9(6):306–11.
10. MacLean B, Sholzberg M, Weyand AC, Lim J, Tang G, Richards T. Identification of women and girls with iron deficiency in the reproductive years. Int J Gynaecol Obstet. 2023;162 Suppl 2:58–67. [DOI: 10.1002/ijgo.14948] [PMID: 37538015]
11. Oyedeji CI, Artz AS, Cohen HJ. How I treat anemia in older adults. Blood. 2024;143(3):205–13. [DOI: 10.1182/blood.2022017626] [PMID: 36827619]
12. Kachroo S, Stewart J, Mahajan P, Silva M. Gastrointestinal complications of NSAIDs: current perspectives. Clin Exp Gastroenterol. 2021;14:431–43. [DOI: 10.2147/CEG.S300208]
13. Campbell HE, Stokes EA, Bargo D, Logan RF, Mora A, Hodge R, et al. Costs and quality of life associated with acute upper gastrointestinal bleeding in the UK: cohort analysis of patients in a cluster randomised trial. BMJ Open. 2015;5(4):e007230. [DOI: 10.1136/bmjopen-2014-007230] [PMID: 25926146]
14. Alzoubaidi D, Lovat LB, Haidry R. Management of non-variceal upper gastrointestinal bleeding: where are we in 2018? Frontline Gastroenterol. 2019;10(1):35–42. [DOI: 10.1136/flgastro-2017-100901] [PMID: 30651955]
15. Bryant RV, Kuo P, Williamson K, Yam C, Schoeman MN, Holloway RH, et al. Performance of the Glasgow-Blatchford score in predicting clinical outcomes and intervention in hospitalised patients with upper GI bleeding. Gastrointest Endosc. 2013;78(4):576–83. [DOI: 10.1016/j.gie.2013.05.003] [PMID: 23790755]
16. Robertson M, Majumdar A, Boyapati R, Chung W, Worland T, Terbah R, et al. Risk stratification in acute upper GI bleeding: comparison of the AIMS65 score with the Glasgow-Blatchford and Rockall scoring systems. Gastrointest Endosc. 2016;83(6):1151–60. [DOI: 10.1016/j.gie.2015.10.021] [PMID: 26515955]

Процена прогностичке вредности Рокалове и Глазгов–Блачфордове скале бодовања код неварикозног крварења из горњег гастроинтестиналног тракта

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САЖЕТАК

Увод/Циљ Крварење из горњег гастроинтестиналног тракта представља озбиљно медицинско хитно стање које захтева брзу процену и интервенцију. Различите скале за стратификацију ризика, укључујући Рокалову и Глазгов–Блачфордову скалу бодовања (ГБС), користе се за предвиђање клиничких исхода као што су морталитет, пријем у јединицу интензивне неге и потреба за трансфузијом крви.

Методе У студију је укључено 199 болесника примљених у нашу болницу због неварикозног крварења из горњег гастроинтестиналног тракта у периоду од 1. октобра 2020. до 1. октобра 2024. године. Забележени су демографски подаци, витални знаци (пулс, систолни и дијастолни крвни притисак), дужина боравка у болници и на интензивној нези, коморбидитети и употреба лекова. За сваког болесника израчунати су Рокалови и ГБС скорови, а њихова предиктивна вредност процењена је анализом сензитивности и специфичности.

Резултати ГБС (*AUC* = 0,887) показао је бољу предиктивну вреност за трансфузију крви у поређењу са Рокаловом скалом (*AUC* = 0,786, $p < 0,001$). Међутим, оба резултата показала су слабу предиктивну способност за пријем у јединицу интензивне неге (*AUC* = 0,624 према 0,605, $p < 0,05$), при чему је Рокалова скала надмашила ГБС. За предвиђање морталитета, оба резултата су се показала слично (Рокал: *AUC* = 0,847, ГБС: *AUC* = 0,837, $p = 0,239$), што не указује на значајну разлику.

Закључак ГБС надмашује Рокалову скалу у предвиђању потребе за трансфузијом крви, док оба скора показују слаб учинак у предикцији пријема у јединицу интензивне неге, при чему је Рокалова скала нешто боља. У предвиђању морталитета, оба скора су упоредива. ГБС је погоднија за процену потребе за трансфузијом, али додатни фактори могу побољшати предикцију пријема у јединицу интензивне неге и морталитета.

Кључне речи: крварење из гастроинтестиналног тракта; Рокалова скала; Глазгов–Блачфордова скала; морталитет; трансфузија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Fertility preservation and oncological outcomes – a retrospective observational pilot study at a university health institution

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SUMMARY

Introduction/Objective Atypical endometrial hyperplasia (AEH) and endometrial intraepithelial neoplasia (EIN) are precursor lesions of endometrioid adenocarcinoma, often detected at an early stage in reproductive-aged women, where fertility-sparing treatment (FST) is crucial.
This study aimed to evaluate diagnostic methods, treatment strategies, and outcomes of FST approaches in Novi Sad, Serbia.

Methods This retrospective observational pilot study evaluated reproductive-aged patients with AEH or FIGO IA1 EIN treated at the University Clinical Center of Vojvodina.

Results A total of 21 reproductive-aged patients (mean age 37.2 ± 4.31 years) were analyzed, with AEH being the most common diagnosis in 14 (67%) patients. Most patients, 19 (90%), were referred for oncofertility consultation ($p < 0.001$), and 17 patients (81%) were recommended fertility-preserving treatments. The Mirena (Bayer AG, Leverkusen, Germany) levonorgestrel-releasing intrauterine system was the most common treatment modality in 14 (67%) patients ($p < 0.001$), especially among those planning future pregnancies, 11 (100%) ($p < 0.05$). Psychological counseling was considered important by 14 patients (67%). Seven patients (33%) achieved pregnancy post-treatment, all resulting in live births, with four spontaneous and three *in vitro* fertilization pregnancies. Younger patients were more likely to plan future pregnancies (mean age 34.2 *vs*. 40 years, $p < 0.001$) and to achieve pregnancy post-treatment (mean age 33.6 *vs*. 39.1 years, $p < 0.001$).

Conclusion Our study confirmed that fertility-preserving treatment for endometrial lesions is effective, aligns with guidelines, and addresses the shift toward younger patients, highlighting the need for uniform protocols and a unified registry.

Keywords: endometrial hyperplasia; endometrial neoplasms; fertility preservation; pregnancy; pregnancy rate

INTRODUCTION

Atypical endometrial hyperplasia (AEH), or endometrial intraepithelial neoplasia (EIN), is a precursor to endometrioid endometrial carcinoma (EEC), the most common gynecological malignancy in developed countries [1]. It results from unopposed estrogen stimulation and is associated with obesity, chronic anovulation, early menarche, late menopause, or estrogen-secreting tumors. AEH is characterized by abnormal gland-to-stroma ratio with atypical cellular features and, if untreated, can progress to EC [2, 3].

Endometrial carcinoma (EC), which accounted for 420,368 cases globally in 2022, represents 4.5% of all female cancers. Its incidence has risen by 130% in the last 30 years, with the highest rates in North America and Eastern Europe [4]. Although most cases occur in women over 50, incidence, especially among those with obesity or who smoke, is increasing. Around 4% of cases affect women under 40, prompting a growing interest in fertility preservation due to early-stage diagnosis and good prognosis. Fertility-sparing treatment (FST) is becoming more common [5–8]. The traditional treatment for EEC typically involves hysterectomy with or without salpingo-oophorectomy [7]. However, for young patients with early-stage endometrial cancer who wish to preserve fertility, several FST approaches can be considered. When evaluating the possibility of an FST approach, the following criteria must be met: the cancer must be EEC G1, and the disease must be confined to the endometrium, confirmed by imaging diagnostics such as pelvic MRI or transvaginal ultrasound [9, 10]. Additionally, there must be no evidence of suspicious or metastatic disease on imaging, and there should be no contraindications for medical therapy. Patients must also be well-informed that FST is not the standard therapy for endometrial cancer. FST approaches are not

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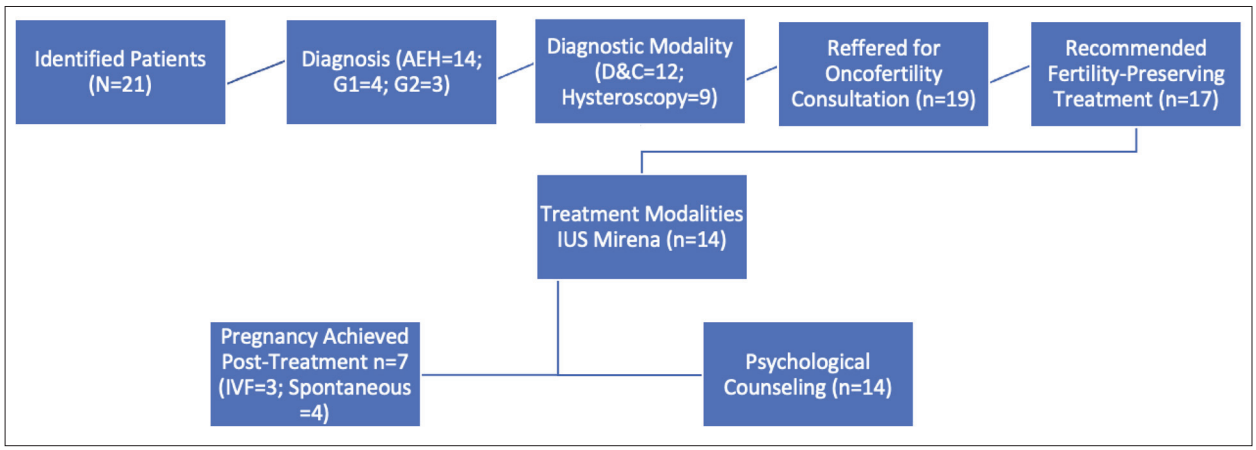

Figure 1. Flow diagram regarding patient selection, diagnostic approaches, treatment modalities, and reproductive outcomes in the cohort

recommended for patients with poorly differentiated EEC, serous EC, clear-cell EC, or carcinosarcoma [10].

The aim of this study was to present and analyze the current practices in diagnosing and treating premalignant and malignant endometrial changes in reproductive-age women wishing to preserve fertility in Novi Sad, Serbia. It also aimed to comprehensively analyze oncological and reproductive outcomes, including the use of assisted reproductive technologies, and to assess the adequacy of the existing treatment approach in line with recommendations from relevant bodies. The study emphasizes the need for a uniform approach to the issue and proposes recommendations for diagnosis and treatment tailored to the specifics of our healthcare system to optimize both oncological and reproductive outcomes.

METHODS

The study was conducted as a retrospective pilot clinical investigation, utilizing data extracted from medical records and relevant anamnesis information at the Clinic of Gynecology and Obstetrics, University Clinical Center of Vojvodina. This included patients with a primary diagnosis of the conditions made at other healthcare institutions, provided they had undergone appropriate pathological verification.

Patients and data collection

The research included a cohort of 21 patients treated between January 2017–May 2023, all diagnosed with pathologically confirmed EC or corresponding premalignant lesions, with the common goal of fertility preservation. As this was a retrospective pilot study aiming to explore feasibility and trends in fertility-preserving management, a formal sample-size calculation was not performed; all eligible cases treated at the institution during the study period were included to maximize data capture and generate preliminary insights. Inclusion criteria required histopathological confirmation of diagnosis, availability of complete medical records, and a documented decision-making process regarding FST. Pathological confirmation was achieved through histological analysis of endometrial biopsy specimens.

The study was conducted in three phases: during the first phase, data relevant to the research objectives were collected from medical records and patient histories; in the second phase, the collected data were subjected to statistical analysis; and in the third phase, the results were compared against current expert guidelines. These guidelines included those of the European Society of Human Reproduction and Embryology (ESHRE), the European Society of Gynecological Oncology (ESGO), and the European Society for Gynecological Endoscopy (ESGE), with the goal of generating tailored recommendations suitable for the context of our healthcare system. A simple flow diagram was added to illustrate patient selection, diagnostic approaches, treatment modalities, and reproductive outcomes in the cohort (Figure 1).

Upon completion of the study, the data were thoroughly verified by the authors, coded for analysis, and entered into a specially designed database. The study results were subsequently presented both in tabular and graphical formats for clearer interpretation and presentation.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were employed to calculate absolute frequencies, corresponding percentages, mean values, and standard deviations, based on the nature of the variables. To assess differences, the Mann–Whitney U test was used for comparing mean values of ordinal variables, while the χ^2 test was applied to evaluate differences between categorical variables. A p-value below 0.05 was interpreted as statistically significant. Data analysis and visualization were performed using Microsoft Office 2021 (Microsoft Corporation, Redmond, WA, USA).

Ethics: The research was performed in line with the Declaration of Helsinki and was approved by the University Clinical Center of Vojvodina Ethics Committee (Document No 6-00-97, May 19, 2023).

RESULTS

The sample consisted of 21 patients (with no missing data), with a mean age of 37.2 ± 4.31 years, ranging 29–43 years. Table 1 shows the frequency of patients according to the pathological findings, with AEH being statistically significantly more common. Out of 21 patients, 14 had AEH, four had EEC G1, and three had EEC G2.

An analysis of the data shows that in twelve patients, the pathological findings were obtained through cervical dilation and curettage (D&C), while in nine patients, the findings were obtained via hysteroscopy (HSC). Although there is a difference in frequencies, it is not statistically significant ($p = 0.664$), indicating a relatively uniform distribution.

Looking at the data in Table 2, it is evident that the majority of patients had already fulfilled the role of a mother prior to diagnosis. The highest number of patients, 19, responded affirmatively to the second question, indicating they were referred to an oncofertility consultation after the diagnosis, and this difference was statistically significant ($p < 0.001$). Although no statistically significant differences were found in the remaining binary questions, it is important to note that FST for uterine preservation was recommended to the majority of patients. More than half of the patients believed that psychological counseling was essential in making decisions about further treatment. Nearly the same number of patients answered both "yes" and "no" when asked about planning pregnancy after treatment. Of the total number of patients, only seven achieved pregnancy after oncological treatment, but all pregnancies resulted in live births.

The majority of patients, 14 in total, were treated with the Mirena (Bayer AG, Leverkusen, Germany) levonorgestrel-releasing intrauterine system (IUS). Oral progestins were recommended for two patients, while surgery was proposed as the therapeutic modality for four patients. As shown, Mirena is a statistically significantly more common choice compared to other options, with this difference being statistically significant ($\chi^2 = 24.5$, $p < 0.001$).

After treatment, among the seven patients who achieved pregnancy, four conceived spontaneously, while three achieved pregnancies through IVF. Regarding the final question from the anamnesis questionnaire – *Was the uterus removed after the achieved pregnancy?* – none of the seven patients who achieved pregnancy had their uterus removed following delivery.

An analysis of the association between the applied therapeutic modality and the pathological findings (Table 3) indicates that in cases of AEH, the most common treatment involved the use of the Mirena IUS, applied in 11 patients. Surgical treatment was chosen for two patients, and oral progestins were recommended for one patient. In cases with pathological findings indicating EEC G1, the most frequent therapeutic modality was also Mirena, used in three patients, while surgery was performed in one case. For pathological findings consistent with EEC G2, surgery was the treatment of choice for two patients, while oral progestins were used in one case. The χ^2 test results show no significant association between the therapeutic modality and the pathological findings. However, due to the small sample size within the categories, this finding should be interpreted with caution.

When analyzing the association between future pregnancy planning and the applied therapy modality (Table 4), it is evident that in the group of patients planning pregnancy, the Mirena IUS was exclusively used as the therapy of choice, applied in 11 cases. Patients treated with medications and/or surgery did not plan pregnancies. According to the results presented in Table 4, patients with the Mirena IUS

Table 1. Distribution of patients according to pathological findings

Pathohistological findings	N (%)	p
AEH	14 (66.7)	0.189
EEC G1	4 (19)	0.007
EEC G2	3 (14.3)	0.001
Total	21 (100)	

AEH – atypical endometrial hyperplasia; EEC – endometrioid endometrial carcinoma

Table 2. Distribution of patient responses to binary questions from the anamnesis questionnaire

Question answered with 'Yes'	N (%)	p
Do you have living children prior to the diagnosis?	14 (66.7)	0.189[a]
When was your diagnosis made, and were you presented to the oncofertility consultation?	19 (90.5)	< 0.001[a]
If presented to the oncofertility consultation, was fertility-preserving treatment for uterine preservation and further oncological treatment recommended?	17 (81)	0.007[a]
Do you think you should have received psychological counseling before making this decision?	14 (66.7)	0.189[a]
Did you plan pregnancy after completing oncological treatment?	10 (47.6)	1.000[a]
Did you achieve pregnancy after oncological treatment?	7 (33.3)	0.189[a]
Did the pregnancy result in a live birth?	7 (33.3)	0.189[a]
Total	21 (100)	

[a]χ^2 test

Table 3. The association between pathological findings and the type of prescribed therapy

Therapeutic modalities	Pathohistological findings						
	AEH	EEC G1	EEC G2	Total	χ^2	df	p
Mirena	11	3	0	14	11.2	8	0.08[a]
Medication	1	0	1	2			
Operation	2	1	2	5			
Total	14	4	3	21			

AEH – atypical endometrial hyperplasia; EEC – endometrioid endometrial carcinoma;
[a]χ^2 test

Table 4. Association between future pregnancy planning and the applied therapy modality

Pregnancy planning	Therapeutic modalities					
	Mirena	Medication	Operation	Total	χ^2	p
Yes	11	0	0	11	9.90	< 0.05[a]
No	4	2	4	10		
Total	15	2	4	21		

[a]χ^2 test

Table 5. Differences in future pregnancy planning by age

Pregnancy planning	N	Mean (years)	Median (years)	SD	SE	U	p
Yes	10	34.2	33.5	3.58	1.13	10.00	< 0.001[b]
No	11	40	39	2.83	0.853		

[b]Mann–Whitney U test

Table 6. Age of patients in relation to the occurrence of pregnancy after oncological treatment

Pregnancy	N	Mean (years)	Median (years)	SD	SE
Yes	7	33.6	33	3.78	1.43
No	14	39.1	39	3.34	0.892

were more likely to plan a pregnancy, and this difference is statistically significant ($\chi^2 = 9.90$, $p < 0.05$).

When examining the association between future pregnancy planning and pathological findings, the results indicate no significant difference in future pregnancy planning based on the patients' pathological findings ($\chi^2 = 93.96$, $p < 0.13$).

The results show that patients with an average age of 34.2 ± 3.58 years planned future pregnancies, while those with an average age of 40.0 ± 2.83 years did not. According to the Mann-Whitney U-test, there is a significant age difference between patients in relation to future pregnancy planning. Patients who planned future pregnancies were significantly younger than those who did not ($U = 10.00$, $p < 0.001$) (Table 5).

We observe that patients who had live births prior to their diagnosis are more likely not to plan a future pregnancy, and this difference is statistically significant ($\chi^2 = 6.11$, $p < 0.01$). There is no significant difference in the frequency of pregnancies following oncological treatment based on the patients' pathological findings ($\chi^2 = 2.20$, $p < 0.33$).

According to the results of the Mann–Whitney U-test, there is a significant difference in patient age concerning the occurrence of pregnancy after oncological treatment. Patients who became pregnant following oncological treatment are significantly younger than those who did not ($U = 14.00$, $p < 0.001$) (Table 6).

DISCUSSION

The selection of patients for FST is critical. Major oncology societies, including the Japan Society of Gynecologic Oncology (JSGO), the ESGO, and the Society of Gynecologic Oncology (SGO), have established criteria for considering FST options in cases of AEH and EEC [11, 12, 13]. They recommend FST for patients with EEC G1 suspected to be confined to the endometrium. The British Gynecological Cancer Society (BGCS) also supports FST for patients with EEC G1 exhibiting superficial myometrial invasion, although for a limited duration [14]. According to joint ESGO, ESHRE, and ESGE guidelines, FST is a viable option for early-stage patients with no metastasis, selected based on comprehensive reproductive potential assessment [13, 14, 15]. These criteria typically apply to patients with AEH or EEC G1 confined to the endometrium, with no myometrial invasion and minimal risk of local invasion or metastatic spread. Recent studies suggest that conservative treatment may be considered on a case-by-case basis for women with early-stage G2 EEC (stage IA) or EEC G2 with minimal myometrial invasion (1–2 mm). These histopathological criteria were previously used to exclude FST [16, 17]. Furthermore, other studies [18, 19, 20] have emphasized the diagnostic challenge in distinguishing AEH from well-differentiated carcinoma, with substantial interobserver variability and frequent underdiagnosis of carcinoma in initial biopsy specimens. Discrepancies between D&C and final hysterectomy pathology are particularly well-documented, often revealing occult carcinoma that was not detected initially. This diagnostic uncertainty can influence treatment decisions and underscores the need for accurate sampling, ideally through hysteroscopically-guided biopsy. Additionally, studies highlight the role of molecular profiling (e.g., POLE, p53, MMR status) and histopathologic risk factors such as lymphovascular space invasion (LVSI), deep myometrial invasion, and tumor grade in determining suitability for conservative management [21]. These parameters are increasingly recognized as critical in identifying patients for whom FST approaches may pose unacceptable oncologic risks. Our study did not include molecular or immunohistochemical analysis, limiting the ability to stratify patients based on more refined risk profiles.

In our study, of the 21 patients considered for FST, 14 had histopathological findings indicating AEH, four had EEC G1, and three had EEC G2, aligning with current guidelines. The selection of candidates for FST should include an assessment of ovarian reserve, anti-Müllerian hormone levels, antral follicle count, FSH levels (on days 2–5), age, and body mass index (BMI), as well as any factors that could affect the patient's ability to carry a pregnancy. Patients with diminished ovarian reserve may still benefit from FST if they opt for oocyte donation.

As women increasingly delay childbirth, with the average age for first-time mothers in the EU rising to 29.4 years in 2019, age remains a significant prognostic factor for fertility, including in patients with AEH or EEC. In our study, the average age was 37.2 years, with the youngest patient being 29 and the oldest 43, which exceeds the upper range of typical age data. Recent meta-analyses show that women with EEC under 35 have the highest chance of live birth rate (30.7%), while those under 40 have a live birth rate of 23% [22].

When examining the relationship between age at diagnosis and plans for future pregnancies, our study found a significant difference: younger patients were more likely to plan for pregnancy ($U = 10.00$, $p < 0.001$). Similarly, patients who achieved pregnancy after oncological treatment were significantly younger ($U = 14.00$, $p < 0.001$). However, there was no significant difference in pregnancy plans based on histopathological findings ($\chi^2 = 93.96$, $p < 0.13$), consistent with current literature.

The diagnostic procedures for EC include endometrial biopsy, with several methods used, including pipelle sampling, cervical dilation and curettage (D/C), and hysteroscopically-guided biopsy. In our study, 12 patients had

histopathological findings from D/C, while nine had results from hysteroscopy. Despite its limitations, D/C has long been favored for obtaining biopsy samples. Studies have shown that D/C samples less than 50% of the endometrial cavity, with up to 10% of lesions missed, particularly focal abnormalities. Consequently, hysteroscopy is now considered the preferred method for endometrial biopsy [13, 23, 24, 25]. For the past 25 years, hysteroscopy and targeted endometrial biopsy have been considered the standard in EC diagnosis. Depending on local findings, a three-step excision technique may be used, ensuring that the obtained material is extracted without introducing the forceps into the operative channel. In cases of atrophic endometrium, bipolar electrodes and scissors are employed for precise lesion removal. Office resectoscopes may also be used to collect larger tissue samples, including subendometrial tissue. A meta-analysis of 65 studies on hysteroscopy's accuracy in diagnosing EC found a sensitivity of 86.4% and specificity of 99.2% [26]. Another meta-analysis, conducted by European and American researchers, confirmed that hysteroscopically-guided biopsy is more accurate than blind biopsy for diagnosing endometrial pathology. Concerns regarding the potential for tumor cell dissemination during hysteroscopy are addressed by studies showing no impact on disease staging or prognosis [27]. Additionally, discrepancies in differentiating atypical hyperplasia from well-differentiated carcinoma, especially when using curettage, are well-documented in the literature.

As with any retrospective observational study, this research has several inherent limitations. Selection bias may have occurred, as only patients who presented to and were treated at a single tertiary center were included, potentially limiting generalizability. Information bias is also possible due to reliance on existing medical records, which may have been inconsistently documented. To mitigate these issues, we included all eligible patients treated during the study period and used standardized criteria for data extraction. Nevertheless, prospective studies with predefined protocols and multicenter collaboration are needed to validate our findings and reduce potential biases. Lastly, this study did not control for potential confounders such as comorbidities, BMI, and hormonal status, all of which may have influenced both treatment selection and reproductive outcomes. Future studies should include a larger, more diverse sample and account for these confounding variables to better identify independent predictors of fertility-preserving treatment success and reproductive outcomes.

CONCLUSION

Our study confirms the effectiveness and safety of FST for premalignant and malignant endometrial lesions, aligned with ESGO, ESHRE, and ESGE guidelines. Younger patients, often without children, increasingly seek preservation, with 4% of cases in women under 40. Hormonal therapy, IUS, and hysteroscopic resection show good outcomes, while post-remission monitoring, assisted reproductive technology, and psychological support are essential. In the absence of large studies, a comprehensive evaluation of health and reproductive potential is key before recommending FST. Our findings highlight the need for a unified registry for monitoring oncofertility and a personalized approach to treatment for each patient.

Conflict of interests: 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. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-up. Int J Gynecol Cancer. 2016;26(1):2–30. [DOI: 10.1097/IGC.0000000000000609] [PMID: 26645990]
3. Markowska A, Chudecka-Głaz A, Pityński K, Baranowski W, Markowska J, Sawicki W. Endometrial Cancer Management in Young Women. Cancers (Basel). 2022;14(8):1922. [DOI: 10.3390/cancers14081922] [PMID: 35454829]
4. Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, et al. Global Cancer Observatory: Cancer Today (version 1.1) [Internet]. Lyon (FR): International Agency for Research on Cancer; 2024 [cited 2025 May 10]. Available from: https://gco.iarc.who.int/today.
5. Gordhandas S, Zammarrelli WA, Rios-Doria EV, Green AK, Makker V. Current Evidence-Based Systemic Therapy for Advanced and Recurrent Endometrial Cancer. J Natl Compr Canc Netw. 2023;21(2):217–26. [DOI: 10.6004/jnccn.2022.7254] [PMID: 36791759]
6. Makker V, MacKay H, Ray-Coquard I, Levine DA, Westin SN, Aoki D, et al. Endometrial cancer. Nat Rev Dis Primers. 2021;7(1):88. [DOI: 10.1038/s41572-021-00324-8] [PMID: 34887451]
7. Terzic M, Norton M, Terzic S, Bapayeva G, Aimagambetova G. Fertility preservation in endometrial cancer patients: options, challenges and perspectives. Ecancermedicalscience. 2020;14:1030. [DOI: 10.3332/ecancer.2020.1030] [PMID: 32419842]
8. Smrz SA, Calo C, Fisher JL, Salani R. An ecological evaluation of the increasing incidence of endometrial cancer and the obesity epidemic. Am J Obstet Gynecol. 2021;224(5):506.e1–e8. [DOI: 10.1016/j.ajog.2020.10.042] [PMID: 33127429]
9. Koh WJ, Abu-Rustum NR, Bean S, Bradley K, Campos SM, Cho KR, et al. Uterine Neoplasms, Version 1.2018, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2018;16(2):170–99. [DOI: 10.6004/jnccn.2018.0006] [PMID: 29439178]
10. 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]
11. Won S, Kim MK, Seong SJ. Fertility-sparing treatment in women with endometrial cancer. Clin Exp Reprod Med. 2020;47(4):237–44. [DOI: 10.5653/cerm.2020.03629] [PMID: 33181010]
12. Ebina Y, Katabuchi H, Mikami M, Nagase S, Yaegashi N, Udagawa Y, et al. Japan Society of Gynecologic Oncology guidelines 2013 for the treatment of uterine body neoplasms. Int J Clin Oncol. 2016;21(3):419–34. [DOI: 10.1007/s10147-016-0981-1] [PMID: 27116188]
13. Rodolakis A, Biliatis I, Morice P, Reed N, Mangler M, Kesic V, et al. European Society of Gynecological Oncology Task Force for Fertility Preservation: Clinical Recommendations for Fertility-Sparing Management in Young Endometrial Cancer Patients. Int J Gynecol Cancer. 2015;25(7):1258–65. [DOI: 10.1097/IGC.0000000000000493] [PMID: 26186070]

14. Sundar S, Balega J, Crosbie E, Drake A, Edmondson R, Fotopoulou C, et al. BGCS uterine cancer guidelines: Recommendations for practice. Eur J Obstet Gynecol Reprod Biol. 2017;213:71–97. [DOI: 10.1016/j.ejogrb.2017.04.015] [PMID: 28437632]
15. Uccella S, Zorzato PC, Dababou S, Bosco M, Torella M, Braga A, et al. Conservative Management of Atypical Endometrial Hyperplasia and Early Endometrial Cancer in Childbearing Age Women. Medicina (Kaunas). 2022;58(9):1256. [DOI: 10.3390/medicina58091256] [PMID: 36143933]
16. Casadio P, La Rosa M, Alletto A, Magnarelli G, Arena A, Fontana E, et al. Fertility-Sparing Treatment of Endometrial Cancer with and without Initial Infiltration of Myometrium: A Single Center Experience. Cancers (Basel). 2020;12(12):3571. [DOI: 10.3390/cancers12123571] [PMID: 33260382]
17. Shan W, Wu P, Yang B, Zhang H, Sun L, Lv Q, et al. Conservative management of grade 2 stage IA endometrial carcinoma and literature review. J Obstet Gynaecol Res. 2021;47(3):984–91. [DOI: 10.1111/jog.14646] [PMID: 33403812]
18. Heremans R, Wynants L, Valentin L, Leone FPG, Pascual MA, Fruscio R, et al. Estimating risk of endometrial malignancy and other intracavitary uterine pathology in women without abnormal uterine bleeding using IETA-1 multinomial regression model: validation study. Ultrasound Obstet Gynecol. 2024;63(4):556–63. [DOI: 10.1002/uog.27530] [PMID: 37927006]
19. Ronsini C, Romeo P, Andreoli G, Palmara V, Palumbo M, Caruso G, et al. Fertility-Sparing Treatments in Endometrial Cancer: A Comprehensive Review on Efficacy, Oncological Outcomes, and Reproductive Potential. Medicina (Kaunas). 2025;61(3):471. [DOI: 10.3390/medicina61030471] [PMID: 40142282]
20. Ronsini C, Mosca L, Iavarone I, Nicoletti R, Vinci D, Carotenuto RM, et al. Oncological outcomes in fertility-sparing treatment in stage IA-G2 endometrial cancer. Front Oncol. 2022;12:965029. [DOI: 10.3389/fonc.2022.965029] [PMID: 36185260]
21. De Rocco S, Buca D, Oronzii L, Petrillo M, Fanfani F, Nappi L, et al. Reproductive and pregnancy outcomes of fertility-sparing treatments for early-stage endometrial cancer or atypical hyperplasia: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2022;273:90–7. [DOI: 10.1016/j.ejogrb.2022.04.019] [PMID: 35526471]
22. Herrera Cappelletti E, Humann J, Torrejón R, Gambadauro P. Chances of pregnancy and live birth among women undergoing conservative management of early-stage endometrial cancer: a systematic review and meta-analysis. Hum Reprod Update. 2022;28(2):282–95. [DOI: 10.1093/humupd/dmab041] [PMID: 34935045]
23. Andrijasevic S, Dotlic J, Arsenovic N, Terzic M. Differences in endometrial carcinoma presentations and characteristics in pre- and postmenopausal women. Srp Arh Celok Lek. 2019;147(11–12):692–8. [DOI: 10.2298/SARH181107055A]
24. Bettocchi S, Ceci O, Vicino M, Marello F, Impedovo L, Selvaggi L. Diagnostic inadequacy of dilatation and curettage. Fertil Steril. 2001;75(4):803–5. [DOI: 10.1016/S0015-0282(00)01792-1] [PMID: 11287038]
25. Goldstein SR. Modern evaluation of the endometrium. Obstet Gynecol. 2010;116(1):168–76. [DOI: 10.1097/AOG.0b013e3181dfd557] [PMID: 20567184]
26. Clark TJ, Voit D, Gupta JK, Hyde C, Song F, Khan KS. Accuracy of hysteroscopy in the diagnosis of endometrial cancer and hyperplasia: a systematic quantitative review. JAMA. 2002;288(13):1610–21. [DOI: 10.1001/jama.288.13.1610] [PMID: 12350192]
27. Di Spiezio Sardo A, Saccone G, Carugno J, Pacheco LA, Zizolfi B, Haimovich S, et al. Endometrial biopsy under direct hysteroscopic visualisation versus blind endometrial sampling for the diagnosis of endometrial hyperplasia and cancer: systematic review and meta-analysis. Facts Views Vis Obgyn. 2022;14(2):103–10. [DOI: 10.52054/FVVO.14.2.023] [PMID: 35781106]

Очување плодности и онколошки исходи – ретроспективна опсервациона пилот-студија у универзитетској здравственој установи

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САЖЕТАК

Увод/Циљ Атипична хиперплазија ендометријума (АХЕ) и интраепителна неоплазија ендометријума (ИНЕ) представљају прекурзорне лезије ендометриоидног аденокарцинома, које се често откривају у раној фази код жена у репродуктивном добу, где је очување плодности од кључног значаја.

Циљ ове студије био је да се процене дијагностичке методе, стратегије лечења и исходи приступа очувању плодности у Новом Саду, Србија.

Методе Ова ретроспективна опсервациона пилот-студија укључила је болеснице у репродуктивном добу са АХЕ или ИНЕ *FIGO IA*1 стадијума, лечене у Универзитетском клиничком центру Војводине.

Резултати Анализирана је 21 болесница у репродуктивном добу (просечна старост 37,2 ± 4,31 година), при чему је најчешћа дијагноза била АХЕ, код 14 (67%) болесница. Већина болесница, њих 19 (90%), упућена је на онкофертилно саветовање ($p < 0,001$), а за 17 болесница (81%) препоручен је третман за очување плодности. Интраутерини систем Мирена био је најчешћи модалитет лечења, примењен код 14 (67%) болесница ($p < 0,001$), посебно код оних које су планирале трудноћу – 11 (100%) ($p < 0,05$). Психолошко саветовање је 14 болесница (67%) сматрало важним. Седам болесница (33%) остварило је трудноћу после лечења, а све су резултирале живим рођењем: четири спонтане и три трудноће помоћу ИВФ. Млађе болеснице чешће су планирале будућу трудноћу (просечна старост 34,2 наспрам 40 година, $p < 0,001$) и остариле је после лечења (просечна старост 33,6 наспрам 39,1 годину, $p < 0,001$).

Закључак Наша студија потврдила је да је третман очувања плодности код лезија ендометријума ефикасан, у складу са препорукама и да одговара на пораст учесталости код млађих болесница, што истиче потребу за уједињеним протоколима и централним регистром.

Кључне речи: ендометријална хиперплазија; ендометријалне неоплазме; очување плодности; трудноћа; стопа трудноће

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Hip function in postoperative physical treatment after trochanteric fractures intramedullary and extramedullary fixation

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SUMMARY

Introduction/Objective Postoperative inpatient physical treatment after trochanteric fracture fixation plays an important role in the hip function restoring and promoting overall recovery. The aim of this work was to compare initial and final hip range of motions and general muscle strength (GMS), as well as gender influence on these scores, during the rehabilitation after intramedullary and extramedullary trochanteric fracture fixation.

Method The outcome of physical treatment (hip flexion, extension and abduction, and GMS) was analyzed in 49 patients after a trochanteric fracture fixation by an intramedullary nail (IM group) or self-dynamizable internal fixator (SIF group).

Results There was significant improvement in ranges of all evaluated motions and GMS after postoperative inpatient physical therapy ($p < 0.05$). There was no significant influence of the gender and age on the observed functional results ($p > 0.05$). Hip flexion was slightly more improved in SIF group, while GMS was slightly more improved in IM group, but close to the level of statistical significance in both the comparisons ($p < 0.2$).

Conclusion Inpatient rehabilitation provides significant recovery of injured hip range of motions and GMS after trochanteric fractures internal fixation. Intramedullary and extramedullary fixation are considered similar in the rehabilitation outcome following these patients. The degree of osteoporosis, being generally higher in female patients, does not significantly affect the final functional results.

Keywords: hip function; trochanteric fracture; self-dynamizable internal fixator; intramedullary nail

INTRODUCTION

Hip fractures are considered serious injuries that can result from falls, various accidents, etc. Trochanteric fractures, as a type of hip fracture, are specific to the elderly population. The frequency of hip fractures increases with age, but trochanteric fractures occur more often in patients over 75 years of age, while neck fractures occur in slightly younger patients [1, 2]. As the age of trochanteric fractures population is often accompanied by various diseases and poor general condition, the treatment is conservative in just a part of patients. However, more than 90% of patients with a trochanteric fracture who are admitted to the authors' hospital are treated surgically by one of the two internal fixation methods: intramedullary nailing (IM) or extramedullary (EM) fixation using a self-dynamizable internal fixator (SIF) (Figure 1) [3–7].

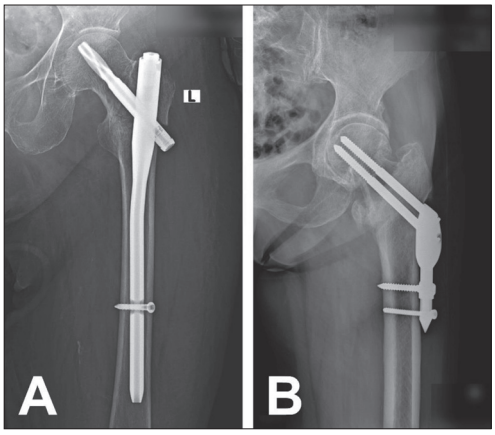

Figure 1. Intramedullary and extramedullary internal fixation methods used in the cases from the study; A – proximal femoral nail antirotation; B – self-dynamizable internal fixator

SIF, developed by Academician Prof. Milorad Mitković, has been in routine clinical use since 1998 [8, 9]. Its advantages include a straightforward surgical technique, standard instrumentation, and reduced X-ray exposure. This implant allows for simultaneous dynamization and compression in two axes, with delayed axial dynamization possible without reoperation. Globally, cephalomedullary nails and EM devices like the sliding hip screw are widely accepted as standard treatments for trochanteric fractures [10, 11, 12]. While intramedullary fixation offers shorter incisions and greater stability for certain fracture types, it typically requires more complex instrumentation and longer fluoroscopy time compared to SIF [7, 10].

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At our institution, trochanteric fractures account for more than 20% of all trauma surgeries, making them the most frequent trauma case. Combined with femoral neck and subtrochanteric fractures, hip fractures constitute nearly 50% of all surgically treated trauma patients [5, 7].

After surgery, patients undergo inpatient physical rehabilitation aimed at early mobilization and restoration of hip function. This process significantly improves clinical outcomes and quality of life [8]. Conservative treatment, in contrast, is linked with higher complication rates and worse outcomes, including thromboembolism due to prolonged immobility [1, 2, 11].

In this paper, the outcome of physical treatment was analyzed after trochanteric fracture internal fixation by IM or SIF method. This work aimed to compare initial and final range of motion and general muscle strength (GMS) in the hip joint, as well as the gender influence on these scores.

METHODS

A total of 49 cases, who had surgery at orthopedics and traumatology department of our hospital and then sent to inpatient rehabilitation department of the same hospital very soon, were analyzed in this research. There were 17 male and 32 female patients, with the average age of 72.4 years. All the patients had a trochanteric fracture, treated surgically by one of the two fixation methods – IM method (IM group, 20 cases) and SIF method (SIF group, 29 cases). Gender distribution of the fixation methods is presented in Table 1. According to the AO classification system, 12 patients (24.5%) had A1 (pertrochanteric two-part fracture), 28 patients (57.1%) had A2 (pertrochanteric multifragmentary fracture), and nine patients (18.4%) had A3 (intertrochanteric) fracture.

All patients received a standard rehabilitation plan that began at seven weeks after surgery, including kinesiotherapy, occupational therapy, magnetotherapy and electrotherapy. Electro and magnetic therapy had been used to induce recovery of injured and frozen tissues, circulation stimulating, swelling decrease, and to have an analgesic effect. Kinesiotherapy and occupational therapy had also played a major role in the rehabilitation, such as range of motion improvement. Rehabilitation lasted three weeks.

The initial assessment (early postoperative status) and the final assessment were performed at the beginning and at the end of the physical therapy (just upon admission and just before discharge from the rehabilitation department). Range of motion, including hip flexion with knee extended and knee flexed, hip extension, hip abduction, and GMS of the operated leg, were measured using a goniometer. GMS was scaled by manual muscle test – applying resistance manually and grading the muscle strength based on the patient's ability to resist as grade 1 (10%), grade 2 (25%), grade 3 (50%), grade 4 (75%), and grade 5 (100%).

A comparison of the parameters was performed between genders too. The data were statistically analyzed by IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA), performing Wilcoxon test, Mann–Whitney U test, and χ^2 test.

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. 37288/17).

RESULTS

There was significant difference in ranges of evaluated motions and GMS next before and next after postoperative physical therapy ($p < 0.05$; Table 2).

The obtained data on the range of motion improvement upon discharge from rehabilitation therapy between IM and SIF groups, but also between genders, indicate that there was no statistically significant difference in any of the modalities ($p > 0.05$; Table 3, Table 4). There was no significant influence of the gender and age on the observed functional results ($p > 0.05$; Table 1, Table 4).

DISCUSSION

Trochanteric fractures are in elderly population mostly sustain as a low-energy trauma, such as a fall at home during daily activities, while in younger population it mostly occurs by high forces affecting directly the hip area [3]. The incidence of trochanteric fractures is constantly increasing due to the increase of general average age in the population, being in accordance with our study, where the average age was about 72 years.

Since there is no significant difference in the final functional results between genders, it can be considered that the degree of osteoporosis, being generally higher in women, does not affect significantly the functional outcome.

As trochanteric fractures mostly occur in the oldest population, a large part of patients never reach their previous level of activity. The success of physical therapy is influenced by the patient's general condition and previous physical activity. To achieve as much progress in recovery after a trochanteric fracture as possible, an extended period of home-based physical therapy is required

Hip function improving due to the applied physical therapy after a trochanteric fracture internal fixation surgery was confirmed by significant increase in range of all hip joint motions tested and in hip area GMS ($p < 0.05$).

Hip flexion increasing was slightly higher in SIF group, but without statistical significance. Here could be considered that the choice between these two fixation methods did not have a general impact on enhancement of hip joint range of motion after physical therapy completion. This also confirms that lateral presence of an EM implant on the proximal part of femur, as it is in SIF, does not interfere significantly with the hip function.

When comparing IM and SIF types of the fixation, it should be mentioned that different surgical approaches are being used. Beside shorter distal incisions, intramedullary fixation of trochanteric fractures is followed by a slightly

Table 1. Distribution of fixation methods by gender

Fixation methods	Male	Female	p
IM	9	11	0.208*
SIF	8	21	
Average age (years)	73.5 ± 6.7	71.8 ± 8.5	0.514**

IM – intramedullary nail; SIF – self-dynamizable internal fixator;
*χ^2 test;
**Mann–Whitney U test

Table 2. Clinical parameters before and after physical therapy, for all patients

Parameters	Beginning of the physical therapy (seven weeks after surgery) (mean ± SD)	End of the physical therapy (10 weeks after surgery) (mean ± SD)	p*
Hip flexion with knee extended (degrees)	34.8 ± 22.9	59.4 ± 25.1	< 0.001
Hip flexion with knee flexed (degrees)	52.3 ± 21.8	21.3 ± 14	< 0.001
Hip extension (degrees)	0	1 ± 2.7	0.015
Hip abduction (degrees)	16.1 ± 8.9	26.6 ± 12.2	< 0.001
General muscle strength	2.1 ± 0.6	2.7 ± 0.6	< 0.001

*Wilcoxon test

Table 3. Improvement of clinical parameters due to applied physical therapy between SIF and IM group

Physical therapy improvement (E-B)*	SIF (mean ± SD)	IM (mean ± SD)	p**
Hip flexion with knee extended (degrees)	26 ± 12.3	22.5 ± 17.7	0.196
Hip flexion with knee flexed (degrees)	24.3 ± 12.7	17 ± 15.1	0.127
Hip extension (degrees)	1.4 ± 3.2	0.5 ± 1.5	0.422
Hip abduction (degrees)	10.2 ± 8.5	11 ± 9.8	0.769
General muscle strength	0.6 ± 0.5	0.7 ± 0.9	0.154

*B – beginning (seven weeks after surgery); E – end (ten weeks after surgery) of the physical therapy;
IM – intramedullary nail; SIF – self-dynamizable internal fixator;
**Mann–Whitney U test

Table 4. Improvement of clinical parameters due to applied physical therapy between genders

Physical therapy improvement (E-B)*	Male (mean ± SD)	Female (mean ± SD)	p**
Hip flexion with knee extended (degrees)	20.3 ± 13.5	26.9 ± 14.9	0.125
Hip flexion with knee flexed (degrees)	23.2 ± 12.5	20.3 ± 14.9	0.855
Hip extension (degrees)	1.2 ± 2.8	0.9 ± 2.7	0.653
Hip abduction (degrees)	9.7 ± 7.2	10.9 ± 9.9	0.880
General muscle strength	0.6 ± 0.7	0.6 ± 0.7	0.970

*B – beginning (seven weeks after surgery); E – end (10 weeks after surgery) of the physical therapy;
**Mann–Whitney U test

longer proximal incision for approaching through the hip abductor muscles. EM fixation of trochanteric fractures includes only one slightly longer incision, distal to greater trochanter, for approaching through the knee extensor muscles. Our study showed that the choice between one of these approaches did not significantly affect the postoperative function of the hip. Even though there was no statistically significant difference in the comparisons between the fixation methods, it should be mentioned that the difference in flexion increase was noticeably closer to the level of statistical significancy ($p < 0.2$), compared to abduction ($p > 0.7$) and extension ($p > 0.4$) increase.

Since the difference on hip range of motion tested and hip GMS was not significantly different between genders, we can conclude that gender did not have an impact on functional outcome of postoperative physical treatment. Lahtinen et al. [13], and Lieberman and Lieberman [14] also concluded that there was no significant difference in final functional results after postoperative physical rehabilitation in trochanteric fractures treatment between genders. In our study, the difference in increase of hip flexion with knee extension (it was higher in females) was noticeably closer to the level of statistical significancy rather in other motion tests. This could possibly be due to the eventual difference in lesser trochanter comminution frequencies among gender groups (these frequencies were not checked in the study), because Van der Sijp et al. [15], reported that a comminution of lesser trochanter can be a considered deaccelerating factor in injured hip flexion restoring.

In almost all patients (47 out of 49 cases), GMS in hip area was either unchanged or improved compared with the level of the strength before physical treatment. Only two patients, treated by IM fixation, had worse level of GMS after the rehabilitation. Range of motions and GMS are the parameters tightly related to both the hip function and gait, as well as quality of life. Tucker et al. [10], Liu et al. [16], and Schemitsch et al. [17], reported that there was no significant difference in functional state of the hip in a trochanteric fracture surgical treatment, when comparing intramedullary and EM [dynamic hip screw (DHS)] fixation. Memon et al. [18] also presented evidence that there was no significant difference in mobility status of the injured hip between the intramedullary [proximal femoral nail (PFN)] and EM (DHS) methods. Prakash et al. [19] reported that the average Harris hip score (HHS) was in average, 5 score points higher in the intramedullary (PFN) group compared to the EM (DHS) group 24 weeks after surgery. Saarenpää et al. [20] compared groups of patients who had undergone trochanteric fracture surgery using the Standardization of Hip Fracture Audit in Europe scoring system for hip functionality, and found that gait scores four months after surgery were higher in the EM (DHS) group than in the intramedullary (Gamma nail) group. In another study by Mitkovic et al. [8], it was reported that no significant difference in HHS and health-related quality of life (SF-12 questionnaire) was noted at least two years after trochanteric fracture surgery between the group with a Gamma nail and the group with a SIF

Our statistical findings about no significant difference in hip functional recovery between IM and EM fixation are in correlation with several recent studies. Clinical trial by Schemitsch et al. [21] found no significant difference in hip function outcomes such as mobility, pain, and daily activities between patients treated with IM and EM methods

over a 12-month follow-up. This aligns closely to the results of our study, denying any notable differences in hip flexion, extension, abduction, and overall muscle strength between the IM and EM groups. Similarly, Prakash et al. [19] reported a slightly higher HHS in the IM group (about five points higher than in EM group) after 24 weeks of rehabilitation. However, they concluded that the difference was not clinically significant. This matches our results about a slightly higher muscle strength IM fixation, though not statistically significant.

Although Bilanovic et al. [12], Gleich et al. [22], Grønhaug et al. [23], La Barbera [24], and Zeelenberg et al. [25] did not all directly evaluate postoperative hip function, they emphasized that both EM and IM are reliable options for fracture stabilization. Their conclusion – that the choice of implant should be determined by the fracture type rather than the inherent superiority of one method – supports our finding that recovery quality appears similar regardless of the implant used. These studies also support the statement that while implant type may have some influence in complex or unstable fractures, it is not a determining factor for outcomes in stable fractures. Micro-movements between fixed fragments of a hip fracture could be expected to be larger in an unstable fracture, reducing the hip motions. In addition to the fracture stability, Zheng et al. [26] found that five more factors (age, history of hypertension, blood transfusion, Parker mobility score, adverse events within 12 months postoperatively, discharge disposition, and time from surgery to weight-bearing) significantly influence one-year functional outcome following hip fracture surgery in geriatric patients. Thus, they likewise found no evidence that the choice between IM and EM fixation significantly influenced functional outcome.

The main limitation of this study is the relatively small number of patients (fewer than 30 in each group). Larger sample sizes would be needed to more precisely explain the appearance of two patients in the intramedullary group with lower GMS after the physical treatment, and if the surgical approach through abductor muscles of the hip, following proximal femur intramedullary fixation technique, was the factor for this GMS decrease. Large sample studies would also clarify whether the hip flexion improvement can still be significantly higher in SIF compared to IM fixation, as well as whether post physical hip flexion with knee extension could still be significantly more improved in women, in trochanteric fractures internal fixation.

CONCLUSION

Trochanteric fractures are common in patients over 70, usually caused by low-energy falls. Osteoporosis increases the risk but does not worsen recovery outcomes when care is properly managed. Surgical fixation is essential, followed by early and intensive rehabilitation. Two main techniques are used: EM fixation, which may allow better hip flexion, and IM fixation, which often leads to slightly better overall mobility. Studies show no major difference in final outcomes between men and women. This suggests that gender and bone density have limited impact on functional recovery. Successful rehabilitation requires a multidisciplinary approach. Early mobilization, tailored physiotherapy, and adequate nutritional and emotional support are crucial for restoring independence and quality of life.

Conflict of interest: None declared.

REFERENCES

1. Takahashi A, Naruse H, Kitade I, Shimada S, Tsubokawa M, Kokubo Y, et al. Functional outcomes after the treatment of hip fracture. PLoS One. 2020;15(7):e0236652. [DOI: 10.1371/journal.pone.0236652] [PMID: 32730298]
2. Hagino H, Furukawa K, Fujiwara S, Okano T, Katagiri H, Yamamoto K, et al. Recent trends in the incidence and lifetime risk of hip fracture in Tottori, Japan. Osteoporos Int. 2009;20(4):543–8. [DOI: 10.1007/s00198-008-0685-0] [PMID: 18633667]
3. Mitkovic MM, Bumbasirevic M, Milenkovic S, Gajdobranski D, Bumbasirevic V, Mitkovic MB. Influence of coronavirus disease 2019 pandemic state of emergency in orthopaedic fracture surgical treatment. Int Orthop. 2021;45(4):815–20. [DOI: 10.1007/s00264-020-04750-3] [PMID: 32728928]
4. Mitkovic MM, Korunovic ND, Milenkovic SS, Stojiljkovic PM, Manic MT, Trajanovic MD. Forces required to dynamize sliding screws in gamma nail and selfdynamizable internal fixator. BMC Musculoskelet Disord. 2024;25(1):271. [DOI: 10.1186/s12891-024-07392-3] [PMID: 38589829]
5. Micic ID, Mitkovic MB, Park IH, Mladenovic DB, Stojiljkovic PM, Golubovic ZB, et al. Treatment of subtrochanteric femoral fractures using Selfdynamisable internal fixator. Clin Orthop Surg. 2010;2(4):227–31. [DOI: 10.4055/cios.2010.2.4.227] [PMID: 21119939]
6. Kostic IM, Mitkovic MM, Mitkovic MB. Treatment of stable and unstable intertrochanteric fractures with selfdynamisable internal fixator (concept of double dynamisation). Vojnosanit Pregl. 2015;72(7):576–82. [DOI: 10.2298/vsp131025068k] [PMID: 26364449]
7. Mitkovic M, Milenkovic S, Micic I, Mladenovic D, Mitkovic M. 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]
8. Mitkovic MM, Milenković SS, Micic ID, Kostić IM, Stojiljkovic PM, Mitkovic MB. Hip function and health-related quality of life in intramedullary and extramedullary internal fixation of trochanteric fractures. Srp Arh Celok Lek. 2020;148(7–8):451–4. [DOI: 10.2298/SARH200301029M]
9. Milenković S, Mitković M, Radenkovic M, Stanojković M, Stanojlović M, Anđelović S, et al. Hirurško lečenje trohanternih preloma metodom duple dinamičke unutrašnje fiksacije. Acta Facultatis Medicae Naissensis. 2002;19(3–4):236–41.
10. Tucker SM, Wee H, Fox E, Reid JS, Lewis GS. Parametric finite element analysis of intramedullary nail fixation of proximal femur fractures. J Orthop Res. 2019;37(11):2358–66. [DOI: 10.1002/jor.24401] [PMID: 31254411]
11. Marks L, Pass B, Knobe M, Volland R, Eschbach D, Lendemans S, et al. Registry for Geriatric Trauma. Quality of life, walking ability and change of living situation after trochanteric femur fracture in geriatric patients – Comparison between sliding hip screw and cephalomedullary nails from the registry for geriatric trauma. Injury. 2021;52(7):1793–800. [DOI: 10.1016/j.injury.2021.05.012] [PMID: 34039468]
12. Bilanovic M, Milenkovic B, Timotijevic S, Tatic M, Milovanovic D. Surgical treatment of peri-implant femoral fractures – case report and literature review. Srp Arh Celok Lek. 2024;152(3–4):196–200. [DOI: 10.2298/SARH230824014P]

13. Lahtinen A, Leppilahti J, Vähänikkilä H, Kujala S, Ristiniemi J, Jalovaara P. No major differences in recovery after hip fracture between home-dwelling female and male patients. Scand J Surg. 2020;109(3):250–64. [DOI: 10.1177/1457496919847932] [PMID: 31088335]
14. Lieberman D, Lieberman D. Rehabilitation following hip fracture surgery: a comparative study of females and males. Disabil Rehabil. 2004;26(2):85–90. [DOI: 10.1080/19653280310001629660] [PMID: 14668144]
15. Van der Sijp MPL, Moonen L, Schipper IB, Krijnen P, du Pré KJ, Niggebrugge AHP. The functional effect of lesser trochanter involvement in hip fractures: A prospective cohort study. Injury. 2020;51(11):2634–9. [DOI: 10.1016/j.injury.2020.09.002] [PMID: 32900470]
16. Liu M, Yang Z, Pei F, Huang F, Chen S, Xiang Z. A meta-analysis of the Gamma nail and dynamic hip screw in treating peritrochanteric fractures. Int Orthop. 2010;34(3):323–8. [DOI: 10.1007/s00264-009-0783-4] [PMID: 19401825]
17. Schemitsch EH, Nowak LL, Schulz AP, Brink O, Poolman RW, Mehta S, et al. Intramedullary nailing vs sliding hip screw in trochanteric fracture management: The INSITE Randomized Clinical Trial. JAMA Netw Open. 2023;6(6):e2317164. [DOI: 10.1001/jamanetworkopen.2023.17164] [PMID: 37278998]
18. Memon K, Siddiqui AM, Khan ZA, Zahoor A. Dynamic hip screw fixation vs. proximal femur nail for unstable pertrochanteric fractures: A comparative analysis of outcomes and complications. J Ayub Med Coll Abbottabad. 2021;33(1):34–8. [PMID: 33774951]
19. Prakash AK, S NJ, Shanthappa AH, Venkataraman S, Kamath A. A comparative study of functional outcome following dynamic hip screw and proximal femoral Nailing for intertrochanteric fractures of the femur. Cureus. 2022;14(4):e23803. [DOI: 10.7759/cureus.23803] [PMID: 35518518]
20. Saarenpää I, Heikkinen T, Ristiniemi J, Hyvönen P, Leppilahti J, Jalovaara P. Functional comparison of the dynamic hip screw and the Gamma locking nail in trochanteric hip fractures: a matched-pair study of 268 patients. Int Orthop. 2009;33(1):255–60. [DOI: 10.1007/s00264-007-0458-y] [PMID: 17943284]
21. Schemitsch EH, Nowak LL, Schulz AP, Brink O, Poolman RW, Mehta S, et al; INSITE Investigators. Intramedullary Nailing vs Sliding Hip Screw in Trochanteric Fracture Management: The INSITE Randomized Clinical Trial. JAMA Netw Open. 2023;6(6):e2317164. [DOI: 10.1001/jamanetworkopen.2023.17164] [PMID: 37278998]
22. Gleich J, Neuerburg C, Linhart C, Keppler AM, Pfeufer D, Kammerlander C, et al. Inferior outcome after unstable trochanteric fracture patterns compared to stable fractures in the elderly. J Clin Med. 2021;10(2):171. [DOI: 10.3390/jcm10020171] [PMID: 33418912]
23. Grønhaug KML, Dybvik E, Matre K, Östman B, Gjertsen JE. Intramedullary nail versus sliding hip screw for stable and unstable trochanteric and subtrochanteric fractures : 17,341 patients from the Norwegian Hip Fracture Register. Bone Joint J. 2022;104-B(2):274–82. [DOI: 10.1302/0301-620X.104B2.BJJ-2021-1078.R1] [PMID: 35094569]
24. La Barbera L, Tanaka A, Berti F, Antonini G, Villa T. Biomechanical comparison between three intramedullary nails and percutaneous compression plate in stable and unstable trochanteric fractures. Clin Biomech (Bristol). 2025;106507. [DOI: 10.1016/j.clinbiomech.2025.106507] [PMID: 40164529]
25. Zeelenberg ML, Plaisier AC, Nugteren LHT, Loggers SAI, Joosse P, Verhofstad MHJ, et al. Extramedullary versus intramedullary fixation of unstable trochanteric femoral fractures (AO type 31-A2): a systematic review and meta-analysis. Arch Orthop Trauma Surg. 2024;144(3):1189–209. [DOI: 10.1007/s00402-023-05138-9] [PMID: 38175213]
26. Zheng W, Cai Q, Zhang C, Chen S, Fu K. One-year functional outcomes following geriatric hip fracture: A prospective cohort analysis. Orthop Surg. 2025;17(7):2130–40. [DOI: 10.1111/os.70081] [PMID: 40421889]

Функција кука у постоперативној рехабилитацији код трохантерних прелома лечених интрамедуларном и екстрамедуларном фиксацијом

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САЖЕТАК

Увод/Циљ Постоперативна физикална терапија након фиксације трохантерних прелома игра важну улогу у обнављању функције кука и укупном опоравку повређених.
Циљ овог рада био је упоређивање почетних и крајњих вредности опсега покрета и укупне мишићне снаге (УМС) у пределу кука, као и утицаја старости и пола болесника на ове параметре током рехабилитационог лечења након интрамедуларне и екстрамедуларне фиксације трохантерних прелома.

Методе Код 49 болесника са трохантерним преломом, лечених фиксацијом интрамедуларним клином (*IM* група) или самодинамизирајућим унутрашњим фиксатором (*SIF* група), анализирани су параметри ефекта физикалног лечења (флексија, екстензија и абдукција кука, као и УМС кука).

Резултати Потврђен је значајан напредак у погледу повећања свих праћених опсега покрета у куку и УМС кука након стационарног физикалног постоперативног лечења ($p < 0,05$). Није било значајног утицаја пола и старости болесника на наведене функционалне параметре ($p > 0,05$). Повећање флексије кука било је нешто веће у *SIF* групи, док је повећање УМС кука било нешто веће у *IM* групи, али само близу прага статистичке значајности ($p < 0,2$).

Закључак Стационарно физикално лечење омогућава значајно побољшање опсега покрета и УМС у повређеном зглобу кука након унутрашње фиксације трохантерних прелома. Интрамедуларна и екстрамедуларна фиксација показују сличан утицај на крајње функционалне резултате кука. Степен остеопорозе, који је генерално већи код жена, не утиче значајно на наведене крајње резултате.

Кључне речи: функција кука; трохантерни прелом; самодинамизирајући унутрашњи фиксатор; интрамедуларна фиксација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Patient-reported behavior and problems related to topical glaucoma medication

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SUMMARY

Introduction Compliance to medicamentous therapy in glaucoma patients if of great importance since it has great effect on clinical outcome.

Methods The descriptive cross-sectional study included patients (303) referred for diagnostic procedures and follow up examinations at the glaucoma outpatient clinic of the University Clinical Center of Serbia University Eye Hospital, in the period from January 2023 to January 2024. The study included glaucoma patients older than 18 years, who have been taking therapy for more than a month. Patients were asked questions about the use of topical antiglaucoma medications. Statistical analysis was performed in SPSS Statistics for Windows, Version 18.0 using t-test. A p-value of less than 0.05 was considered statistically significant.

Results 303 glaucoma patients were included in the study, of which 120 (39.6%) were men, and 183 (60.4%) women. The average age was 67.4 ± 12.6 years (19–92). The average duration of therapy was 7.1 ± 5.8 years (one month to 30 years). The largest number of patients, 177 of them (58.4%) stated that they regularly use the prescribed therapy.

Conclusion Our study showed a high degree of reported compliance of the tested patients, which is significant for the correct choice of therapeutic modalities in the future, which would slow down the progression of this incurable disease.

Keywords: compliance; topical antiglaucoma therapy; drop instillation technique

INTRODUCTION

Chronic diseases require long-term and persistent use of medications [1]. The course of the disease, in addition to choosing the optimal therapeutic protocol, is also decisively influenced by compliance, which is defined as "whether the patient uses the drug as prescribed by the doctor" or "the degree or extent of conformity to the recommendations about day-to-day treatment by the provider with respect to the timing, dosage, and frequency" [2]. In chronic ophthalmological diseases, for which there are different therapeutic modalities, such as glaucoma, topical drug therapy is often the first choice. It has been proven an effective and non-invasive solution, available to everyone and relatively simple to use [3, 4]. Whenever medical therapy for chronic diseases is discussed, the concept of compliance is inevitably mentioned.

The aim of this study is to assess glaucoma patients' compliance and to evaluate and point out the most common errors when applying eye drops.

METHODS

The prospective cross-sectional study included 303 patients referred for diagnostic procedures and follow up examinations at the glaucoma outpatient clinic of the University Clinical Center of Serbia, University Eye Hospital, from January 2023 to January 2024.

The study included glaucoma patients over 18, who have been taking topical antiglaucoma therapy for more than three months, which was inclusion criteria. The exclusion criteria included persons under 18 years of age, and those who are not being treated for glaucoma. Respondents were asked questions about the use of topical antiglaucoma medications. The questionnaire consisted of questions related to the patient disease (length of treatment), therapy (number of drugs, regularity of use) and technique of applying eye drops. The questions asked are recommended by the European Glaucoma Society for assessing compliance with glaucoma medication therapy (https://www.eugs.org/educational_materials/6). Descriptive statistics of participant demographics were computed, including means, standard deviations, frequencies, and percentages and for three independent variables (age, gender, number of drops). Multiple logistic regression analysis was then used to examine how the preselected variables were associated with compliance. The obtained data were processed in the SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA) program. A p-value of less than 0.05 was considered statistically significant.

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Ethics: This study was undertaken according to the tenets of the Helsinki Declaration, and approved by the Ethics Committee of the University Clinical Center of Serbia (278/22). All participants received oral and written study-related information, and each participant provided written informed consent.

RESULTS

303 glaucoma patients were included in the study, of which 120 (39.6%) were men, and 183 (60.4%) women. The results are presented in Table 1. The majority of patients (40.3%), were on monotherapy, and the fewest tested patients had four drugs in their glaucoma therapy (10.2%). Of all patients tested, 177 (58.4%) stated that they regularly use the prescribed therapy. As many as 224 (73.9%) patients stated that they take the therapy themselves, 42 of them (13.9%) require the help of another person, while 37 patients (12.2%) stated that they apply it both by themselves and with the help of others. As for the position in which patients administer drops, it is most often the sitting position (45.5%), and the least common is the standing position (23.5%). A total of 225 patients (74.3%) instill one drop in the eye, 65 (21.4%) instill two drops on average during application, while 13 patients (4.3%) instill three or more drops in each eye. Less than half of the respondents were given a demonstration of instillation by an ophthalmologist or nurse (132 patients or 43.6%). Most patients wash their hands before instilling eye drops (85.2%), but also most of them do not have the habit of pressing the lacrimal ducts after instilling the drops (78.5%). Our results show no statistically significant difference in compliance in relation to age ($p = 0.627$) and gender ($p = 0.512$) in our study group. In addition, the number of medications did not affect the regularity of administration ($p = 0.514$), nor did the demonstration of the technique of applying the drops. No relationship between tested independent variables and reported compliance was found. Results are shown in Table 2.

Table 1. Characteristics of the study population

Gender	n (%)
Male	120 (39.6%)
Female	183 (60.4%)
Regular usage of eye drops	
Yes	177 (58.4%)
No	126 (41.6%)
Number of topical medications	
1	122 (40.3%)
2	88 (29%)
3	62 (20.5%)
4	31 (10.2%)
Drop instillation technique	
By themselves	224 (73.9%)
With assistance	42 (13.9%)
By themselves and with assistance	37 (12.2%)
Position	
Sitting	138 (45.5%)
Lying	94 (31%)
Standing	71 (23.5%)
Demonstrated technique	
Yes	132 (43.6%)
No	171 (56.4%)
Closing eyes	
Yes	170 (56.1%)
No	133 (43.9%)
Press lacrimal duct area	
Yes	65 (21.5%)
No	238 (78.5%)
Washing hands	
Yes	258 (85.2%)
No	45 (14.8%)
Tip contamination	
Yes	39 (12.8%)
No	264 (87.2%)
Instilling drop straight to the eye	
Yes	241 (79.5%)
No	62 (20.5%)
Number of drops per one instillation	
1	225 (74.3%)
2	65 (21.4%)
3	13 (4.3%)
Total	303 (100%)

Table 2. Results

Age	Independent variables	p-value
Above 40 years	Age	0.78
Above 50 years		0.64
Above 60 years		0.72
Above 70 years		0.45
Above 80 years		0.57
	Gender: female	0.51
	N of glaucoma medications	0.88

DISCUSSION

Mutual trust between doctor and patient is very important for all diseases, but the concept of trust in the case of glaucoma has a multifaceted meaning. Glaucoma is a chronic neurodegenerative disease in which responsibility for the disease outcome is shared between the doctor and the patient. The responsibility for timely diagnosis and adequate therapy rests with the doctor, but responsibility related to treatment equals regular instillation of eye drops, and is practically exclusively a matter of the patient. Glaucomatologists must be convinced that their patients take seriously the need to be treated, that is, to instill their eye drops multiple times every day. Also, very often it is necessary to reassure that proper treatment is on. It is precisely from there that the issues of compliance and adherence in glaucoma arise.

The concepts of compliance and adherence first emerged in early 1970s of the 20th century [5]. In glaucoma, these terms became more visible with the appearance of newer types of topical antiglaucoma medications [6]. Adherence is a term that describes the prevalence of the use of the prescribed medicine at different points in time

and directly depends on the active role of the subject – the patient must understand what disease he is suffering from, how it should be treated and believe in the effectiveness of the therapy. According to current definitions, compliance is the degree to which the patient "complies," or obeys the instructions related to his treatment. In the very name of this term, passivity is expressed, and the joint fight of doctor and patient against chronic disease is neglected, which bothers many physicians. That is why lately the active role that the patient must take in the treatment of his chronic disease is more often pointed out. The responsibility for increasing the degree of compliance and adherence lies largely with the prescribing physician. The problem of compliance in glaucoma is complex for various reasons [7]. First, it is difficult for patients to recognize the importance of regular application of therapy for slowly progressing diseases because they do not disturb the quality of life for a long period of time. Another reason is that after receiving therapy, there may be no subjective improvement of the clinical symptoms. Non-compliance with therapeutic protocols leads to further progression of the disease, change of the previously prescribed therapy, and even recommendations of unnecessary surgical procedures, all of which burdens the healthcare system [8]. Studies show that the percentage of non-compliant behavior in glaucoma is 5–80%. Ha et al. [7] came to the conclusion that 50% of patients stop using drops during the first six months of use, and after a year that percentage drops to 33%. We investigated self reported glaucoma medication compliance in our study, and the results on limited sample size show that 58.4% of glaucoma patients use antiglaucoma medications on regular bases. There are several ways to check our patient's compliance and adherence. For quick orientation in everyday clinic work, one of the indicators is whether the patient knows the names of the drugs he instills, whether he knows the instillation regime and whether he instilled the drops on the day he comes for the check-up. Besides patient self-reporting, other traditional and least expensive methods of monitoring medication adherence is analyzing health insurance claims data or pharmacy claims data [9, 10]. However, it is clear that there is potential inaccuracy in traditional compliance and adherence monitoring methods, so more objective methods such as digital sensor monitoring systems have been developed, and are considered the "gold standard" for assessing medication adherence. One of the first, more modern and precise way of monitoring adherence was designed in the 1990s, when the eye drop bottle with a C-cap was designed [11]. The shortcoming of this compliance monitoring model was that it could only be applied to one type of antiglaucoma drug and that it was not implemented among ophthalmologists, nor among glaucoma patients. However, this endeavor to improve compliance led to its increase from 54% to 73%, as determined in studies conducted on this issue. Recent advances in electronic adherence monitoring include E-Novelia (Nemera, Lyon, France), KaliDrop (S-LAB Sp. z.o.o, Mirkow, Poland), eye drop bottle motion sensor system which are all used with variable implementation among patients [12–17]. Unfortunately, each of these systems entails a significant increase in glaucoma treatment costs, making it financially difficult to implement, so it is necessary to reorient to the more affordable systems and methods. In addition to financial obstacles for the implementation of modern electronic models for monitoring the compliance and adherence of glaucoma patients, there are also a number of ethical issues that arise on this topic, especially concerns about patient privacy and data sharing.

When discussing the technique of instilling eye drops, it is important to demonstrate the patients the correct way how to do it, even several times. These data vary by region and depend on the organization of the ophthalmology service [18]. We tried to emphasize the training of patients, i.e., the fact that it is important to point out to every patient, at the beginning of glaucoma treatment, the importance of regular eye drops usage and to show them in a practical way how to do it correctly. Almost half of our respondents (43.6%) stated that they were shown how to put in their eye drops; in 72% of cases, it was shown by a doctor, and in 28% by a nurse. A way to indirectly evaluate patient compliance is to estimate how many drops are actually instilled into the eye in each instillation, and 225 (74.3%) of our tested subjects self-report that they instill one drop in the eye, 65 (21.4%) instill two drops on average during application, while 13 patients (4.3%) instill three or more drops in each eye. In other studies, these data vary from 48.1% to 51% of patients who dispensed more than one necessary drop for single application.

Due to the prevalence and complexity of the compliance problem, intensive work is being done to develop methods that would objectively determine its degree. This is possible through questionnaires, interviews or diary keeping, video analysis of the drip procedure, or direct observation of the pouring technique [19]. In theory, electronic dosage monitoring represents the most objective method. However, it does not provide information on whether the drop of the medicine actually got into the eye, so it must be supplemented with a video recording or supervision of the examinee during instillation. On the other hand, the measurement of intraocular pressure is also not reliable because even a couple of doses before the scheduled control will show values within the reference range [20]. Since it is difficult to find an objective method which will provide an objective assessment of compliance, and it is even more demanding to implement it, questionnaires are often used. They are based on the self-assessment of the respondents and therefore, in 9–14% of cases, they show a deviation from the results of the objective parameters. Nevertheless, they are considered sufficiently informative and are today the most widespread way of evaluating compliance [21, 22].

Our strong belief is that the most important way to fight for greater compliance and adherence is to educate patients. Knowledge about the chronic disease, its course, methods of treatment and consequences of non-treatment can lead to increase of compliance. Although some authors believe that the patient who is familiar with the disease is the one who better adheres to therapy, others emphasize discipline and the help of family and environment [17, 18]. Other strategies include the use of as few drugs as possible

– monotherapy, or the use of fixed combinations of drugs [19, 20, 21]. When it comes to ophthalmological diseases, eye drop instillation technique is of crucial importance in successful treatment, since the delivery of medicine is different than just swallowing a pill [22, 23]. Studies have shown that less than 10% are able to do all the steps adequately. We were particularly interested in data on the technique of instillation of drops in our respondents, and our results show that majority of patients instill the drops themselves (73.9%), most of them in sitting position (31%), and almost half of them (56.1%) have the habit of closing their eyes after putting eye drops in. Our results show no statistically significant difference in compliance in relation to age ($p = 0.627$) and gender ($p = 0.512$) in our study group. In addition, the number of medications did not affect the regularity of administration ($p = 0.514$), nor did the demonstration of the technique of applying the drops.

As for the definition of a compliant patient, there is no standardized questionnaire to determine it, so there are differences among authors regarding this issue. Some authors consider that a patient who misses one dose during the month or week is already non-compliant, while Wolfram et al. [24] include in this category those who miss two or more doses per week. Although in many studies the subject is either compliant or not, Moore et al. [17] divided compliance into three categories: low, medium and complete. All this further complicates the interpretation of data and the comparison of the results of different studies.

Compliance is largely determined by factors related to the patient (age, gender, education), by socioeconomic conditions, the health system, the disease itself, and the type of therapy [25, 26, 27]. The results of our study did not show a relationship between compliance and age and gender of the patient, which is confirmed by previous research [22, 24]. Such results are due to the fact that the elderly population makes up the majority of patients on antiglaucoma therapy, which is also the case in this study. However, when looking only at patients on prostaglandin analogues, Erras et al. [10] concluded that a lower degree of compliance is present in patients under 50 and over 80 years of age. The conclusions of some studies suggest that a larger number of prescribed drops doubles the chance that the patient will miss to administer the therapy [7]. In our sample, the number of medications did not influence the compliance, which is also confirmed by the study by Kang et al. [27].

The economic or financial aspect of glaucoma treatment cannot be ignored, i.e., the impact that high compliance and adherence have on the price of glaucoma treatment. The conclusions of several studies on chronic disease management are that there are strong economic arguments for investing in multidisciplinary interventions that improve patient compliance and adherence [28]. Precise data on how much glaucoma treatment "costs," are unknown for Serbia, but certainly the entire price of "successful" treatment is much lower than the price we would have to pay for a person who is legally blind due to glaucoma [29].

Another confirmation of the thesis that our patients suffering from glaucoma require not only timely diagnosis and treatment, but also continuous support in terms of maintaining adherence and compliance is obtained from the results of the study published by Killeen et al. [30] in which a significant drop in adherence and compliance was obtained after the cessation of a motivational glaucoma coaching intervention [30]. So, in order to achieve the best outcome of glaucoma treatment, it is necessary for our patients to be constantly in some sort of support program. This may mean the need to discuss the use of eye drops with our patients at each check-up and constantly emphasize the importance of good compliance and adherence.

CONCLUSION

Our results show that the majority of our patients adhere to the ophthalmologist's advice regarding regular instillation of anti-glaucoma drops. For a better insight into this problem, it needs to be processed on a much larger sample. We consider our study as an excellent opportunity to point out again the importance of compliance in glaucoma and the need to spend more time with our patients, in order to explain to them in detail, and even more than once, the nature of their disease and the method of treatment.

Conflict of interest: None declared.

REFERENCES

1. Božić M, Milenković M, Pavlović DM, Stamenković M, Pavlović AM. Vitamin B1, eye and brain, Srp Arh Celok Lek. 2022;150(3–4):233–7. [DOI: 10.2298/SARH210929019B]
2. Quaranta L, Novella A, Tettamanti M, Pasina L, Weinreb RN, Nobili A. Adherence and Persistence to Medical Therapy in Glaucoma: An Overview. Ophthalmol Ther. 2023;12(5):2227–40. [DOI: 10.1007/s40123-023-00730-z] [PMID: 37311908]
3. Kinast RM, Sanchez FG, Rees JP, Yeh K, Gardiner SK, Dawes J, et al. Eye Drop Adherence With an Eye Drop Bottle Cap Monitor. J Glaucoma. 2023;32(5):369–73. [DOI: 10.1097/IJG.0000000000002166] [PMID: 37053080]
4. Hallaj S, Chuter BG, Lieu AC, Singh P, Kalpathy-Cramer J, Xu BY, et al. Federated Learning in Glaucoma: A Comprehensive Review and Future Perspectives. Ophthalmol Glaucoma. 2024;8(1):92–105. [DOI: 10.1016/j.ogla.2024.08.004] [PMID: 39214457]
5. While A. Medication adherence: understanding the issues and finding solutions. Br J Community Nurs. 2020;25(10):474–9. [DOI: 10.12968/bjcn.2020.25.10.474] [PMID: 33030359]
6. Moore SG, Richter G, Modjtahedi BS. Factors Affecting Glaucoma Medication Adherence and Interventions to Improve Adherence: A Narrative Review. Ophthalmol Ther. 2023;12(6):2863–80. [DOI: 10.1007/s40123-023-00797-8] [PMID: 37698824]
7. Ha A, Jang M, Shim SR, Kim CY, Chang IB, Kim YK. Interventions for Glaucoma Medication Adherence Improvement: A Network Meta-analysis of Randomized Controlled Trials. Ophthalmology. 2022;129(11):1294–304. [DOI: 10.1016/j.ophtha.2022.06.025] [PMID: 36028393]
8. Ramesh PV, Parthasarathi S, John RK. An exploratory study of compliance to anti-glaucoma medications among literate primary glaucoma patients at an urban tertiary eye care center in South India. Indian J Ophthalmol. 2021;69(6):1418–24. [DOI: 10.4103/ijo.IJO_2008_20] [PMID: 34011712]

9. Vinod K, Sidoti PA. How glaucoma care changed for the better after the pandemic. Curr Opin Ophthalmol. 2022;33(2):59–66. [DOI: 10.1097/ICU.0000000000000812] [PMID: 34698672]
10. Erras A, Shahrvini B, Weinreb RN, Baxter SL. Review of glaucoma medication adherence monitoring in the digital health era. Br J Ophthalmol. 2023;107(2):153–9. [DOI: 10.1136/bjophthalmol-2020-317918] [PMID: 33858837]
11. Ogata G, Yoneda M, Ogawa R, Hanawa A, Asai K, Yamagishi R, et al. Real-Time Measurement of Antiglaucoma Drugs in Porcine Eyes Using Boron-Doped Diamond Microelectrodes. ACS Sens. 2024;9(2):781–8. [DOI: 10.1021/acssensors.3c02088] [PMID: 38244038]
12. Abaidoo B, Mashige KP, Govender-Poonsamy P, Tagoe NN, Essuman VA, Adam SY. Glaucoma Disease-Specific Adherence Measurement Tools Validated for Measuring Adherence to Glaucoma Medications: A Systematic Review. Health Sci Rep. 2025;10;8(2):e70427. [DOI: 10.1002/hsr2.70427] [PMID: 39931261]
13. Gatwood J, Brooks C, Meacham R, Abou-Rahma J, Cernasev A, Brown E, et al. Facilitators and Barriers to Glaucoma Medication Adherence. J Glaucoma. 2022;31(1):31–6. [DOI: 10.1097/IJG.0000000000001965] [PMID: 34772874]
14. Nishimura K, Tabuchi H, Nakakura S, Nakatani Y, Yorihiro A, Hasegawa S, et al. Evaluation of Automatic Monitoring of Instillation Adherence Using Eye Dropper Bottle Sensor and Deep Learning in Patients With Glaucoma. Transl Vis Sci Technol. 2019;8(3):55. [DOI: 10.1167/tvst.8.3.55] [PMID: 31293810]
15. Payne N, Gangwani R, Barton K, Sample AP, Cain SM, Burke DT, et al. Medication Adherence and Liquid Level Tracking System for Healthcare Provider Feedback. Sensors (Basel). 2020;20(8):2435. [DOI: 10.3390/s20082435] [PMID: 32344754]
16. Aguilar-Rivera M, Erudaitius DT, Wu VM, Tantiongloc JC, Kang DY, Coleman TP, et al. Smart Electronic Eyedrop Bottle for Unobtrusive Monitoring of Glaucoma Medication Adherence. Sensors (Basel). 2020;20(9):2570. [DOI: 10.3390/s20092570] [PMID: 32366013]
17. Moore SG, Richter G, Modjtahedi BS. Factors Affecting Glaucoma Medication Adherence and Interventions to Improve Adherence: A Narrative Review. Ophthalmol Ther. 2023;12(6):2863–80. [DOI: 10.1007/s40123-023-00797-8] [PMID: 37698824]
18. Tripathi RK, Shah A, Jalgaonkar SV, Kerkar S. Evaluation of antiglaucoma drug treatment awareness and patient-reported medication adherence: Determinants of glaucoma management. J Postgrad Med. 2023;69(3):146–52. [DOI: 10.4103/jpgm.jpgm_905_22] [PMID: 37313943]
19. Mehuys E, Delaey C, Christiaens T, Van Bortel L, Van Tongelen I, Remon JP, et al. Eye drop technique and patient-reported problems in a real-world population of eye drop users. Eye (Lond). 2020;34(8):1392–8. [DOI: 10.1038/s41433-019-0665-y] [PMID: 31690823]
20. Stingl JV, Greslechner R, Brandl C, Heid IM, Hoffmann EM, Pfeiffer N, et al. Bewusstsein für eine Glaukomerkrankung in der Bevölkerung [Awareness for glaucoma in the general population]. Ophthalmologie. 2023;120(11):1088–97. [DOI: 10.1007/s00347-023-01943-0] [PMID: 37847376]
21. Usgaonkar U, Zambaulicar V, Shetty A. Subjective and objective assessment of the eye drop instillation technique: A hospital-based cross-sectional study. Indian J Ophthalmol. 2021;69(10):2638–42. [DOI: 10.4103/ijo.IJO_3333_20] [PMID: 34571604]
22. Zaharia AC, Dumitrescu OM, Radu M, Rogoz RE. Adherence to Therapy in Glaucoma Treatment-A Review. J Pers Med. 2022;12(4):514. [DOI: 10.3390/jpm12040514] [PMID: 35455630]
23. Sanchez FG, Mansberger SL, Newman-Casey PA. Predicting Adherence With the Glaucoma Treatment Compliance Assessment Tool. J Glaucoma. 2020;29(11):1017–24. [DOI: 10.1097/IJG.0000000000001616] [PMID: 32740508]
24. Wolfram C, Stahlberg E, Pfeiffer N. Patient-Reported Nonadherence with Glaucoma Therapy. J Ocul Pharmacol Ther. 2019;35(4):223–8. [DOI: 10.1089/jop.2018.0134] [PMID: 30897019]
25. Taneja M, Chappidi K, Harsha Ch SNS, Richhariya A, Mohamed A, Rathi VM. Innovative bulls eye drop applicator for self-instillation of eye drops. Cont Lens Anterior Eye. 2020;43(3):256–60. [DOI: 10.1016/j.clae.2019.11.010] [PMID: 31813766]
26. Alwazae M, Alhumud A, Aldarrab A, Hemid AB, AlHassan RA, AlAdel F, et al. Encounter glaucoma decision Aid trial. Eur J Ophthalmol. 2023;33(1):291–6. [DOI: 10.1177/11206721221093020] [PMID: 35975303]
27. Kang JM, Chatterjee A, Rosdahl JA, Bosworth HB, Woolson S, Olsen M, et al. Health Literacy and Success with Glaucoma Drop Administration. Ophthalmol Glaucoma. 2022;5(1):26–31. [DOI: 10.1016/j.ogla.2021.05.004] [PMID: 34052458]
28. Newman-Casey PA, Rhee DJ, Robin AL, Mansberger SL. Patient Challenges with Glaucoma Eye Drops: A Need to Identify Nonadherence and Facilitate Appropriate Support and Disease Management. Ophthalmol Glaucoma. 2025;8(4):327–30. [DOI: 10.1016/j.ogla.2024.12.002] [PMID: 39818641]
29. Ćoškov AD, Savanović DR, Todorović NB, Lalić-Popović MN, Tomić NZ, Milijašević BZ. Hospital Drug Consumption And Treatment Costs of The Glaucoma Therapy in The Republic of Serbia. Pharmacology. 2024;11(3):1499–508. [DOI: 10.5937/hpimj2403499C]
30. Killeen OJ, Niziol LM, Cho J, Heisler M, Resnicow K, Darnley-Fisch D, et al. Glaucoma Medication Adherence 1 Year after the Support, Educate, Empower Personalized Glaucoma Coaching Program. Ophthalmol Glaucoma. 2023;6(1):23–8. [DOI: 10.1016/j.ogla.2022.08.001] [PMID: 35953021]

Понашање пацијената и проблеми повезани са локалном медикаментном терапијом глаукома

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САЖЕТАК

Увод Придржавање медикаментне терапије код пацијената са глаукомом је важно јер има велики утицај на клинички исход лечења.

Методе У дескриптивну студију пресека укључени су пацијенти (303) упућени на лечење у амбуланту за глауком Клинике за очне болести Универзитетског клиничког центра Србије, у периоду од јануара 2023. године до јануара 2024. године. У студију су укључени пацијенти старији од 18 година, који су терапију узимали дуже од месец дана. Испитаницима су постављана питања везана за примену топикалне антиглаукомне терапије. Добијени подаци обрађени су у статистичком програму *SPSS*, верзија 18.0 (*SPSS Inc*., Чикаго, ИЛ, САД) помоћу *t*-теста. Статистички значајном је сматрана *p*-вредност мања од 0,05.

Резултати Од 303 испитаника, 120 (39,6%) били су мушкарци, а 183 (60,4%) жене. Просечна старост износила је 67,4 ± 12,6 година (19–92). Просечно трајање терапије било је 7,1 ± 5,8 година (месец дана до 30 година). Највећи број пацијената, њих 177 (58,4%), навео је да редовно укапава прописану терапију.

Закључак Наша студија показала је висок степен пријављене комплијансе тестираних пацијената, што је значајно за правилан избор терапијских модалитета у будућности, којима би се успорила прогресија ове неизлечиве болести.

Кључне речи: комплијанса; топикална антиглаукомна терапија; техника укапавања капи

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Successful heart transplantation following long-term left ventricular assist device support in a patient with peripartum cardiomyopathy – the first case in Serbia

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SUMMARY

Introduction Peripartum cardiomyopathy (PPCM) is a rare and potentially life-threatening cause of heart failure occurring during late pregnancy or the early postpartum period. While medical therapy remains the first-line treatment, refractory or end-stage heart failure may necessitate surgical intervention, including mechanical circulatory support or heart transplantation.

Case outline We report the first case in Serbia of a young woman with PPCM who successfully underwent left ventricular assist device (LVAD) implantation followed by orthotopic heart transplantation. A 25-year-old woman presented with severe biventricular heart failure 3.5 months postpartum. Despite optimal medical management, her condition deteriorated, necessitating LVAD implantation as bridge-to-transplant therapy. She remained on LVAD support for five years, achieving excellent quality of life, until a suitable donor heart became available. Heart transplantation was performed using the bicaval technique, with no perioperative complications or early graft rejection. The patient was discharged in stable condition.

Conclusion This case highlights the feasibility and success of a multidisciplinary approach combining LVAD support and heart transplantation in managing advanced PPCM. Timely recognition of disease progression and individualized surgical planning are critical to achieving favorable outcomes in this high-risk population.

Keywords: peripartum cardiomyopathy; left ventricular assist device; heart transplantation; mechanical circulatory support; bridge-to-transplant

INTRODUCTION

Peripartum cardiomyopathy (PPCM) is a rare, idiopathic form of cardiomyopathy that presents as heart failure (HF) during the third trimester of pregnancy or within the first five months postpartum. It is characterized by left ventricular (LV) systolic dysfunction with an ejection fraction (EF) of less than 45%, occurring in women without pre-existing cardiovascular disease or other identifiable causes of HF [1, 2, 3].

The standard management of PPCM primarily involves medical therapy. However, in cases of refractory or end-stage HF, surgical interventions may be required, with cardiac transplantation often regarded as the definitive treatment [4]. The limited availability of donor hearts, however, constrains this option. In response, the development of safe, durable mechanical circulatory support (MCS) devices has enabled bridge-to-transplant (BTT) therapy to become an established standard of care for patients awaiting heart transplantation [5, 6].

We report the first case in Serbia of a patient with pregnancy-related HF who underwent initial left ventricular assist device (LVAD) implantation, followed by successful heart transplantation [7].

CASE REPORT

A 25-year-old woman with no significant prior medical history, except for gestational diabetes and gestational hypertension, developed malaise and intermittent cough during the first postpartum month. She initially disregarded these symptoms, but at 3.5 months postpartum, she presented with progressive dyspnea, palpitations, and lower-extremity edema, prompting admission to a regional hospital.

On examination, she was hypotensive (blood pressure 80/50 mmHg), with bilateral lower-limb edema, bilateral basal inspiratory crackles, and a systolic murmur at the apex on auscultation. Transthoracic echocardiography revealed LV dilation (end-diastolic diameter 63 mm, end-systolic diameter 58 mm), severely reduced LV EF (15%), grade 3 mitral regurgitation, and a right-sided pleural effusion. PPCM

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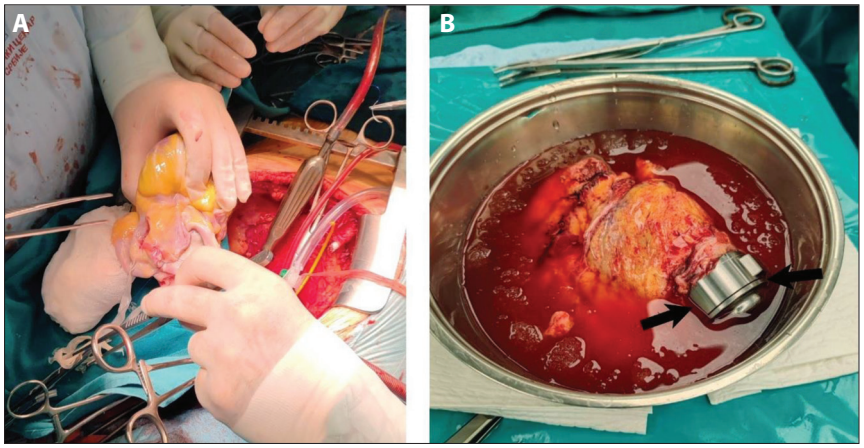

Figure 1. A – donor's heart final preparation; B – recipient's heart with left ventricular assist device (marked with black arrows) after removal

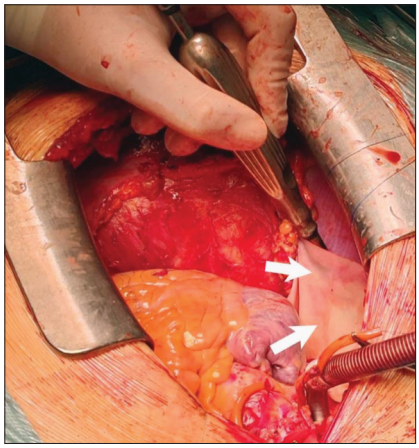

Figure 2. Reconstruction of pericardium with bovine patches (marked with white arrows)

was suspected and the patient was treated with fluid restriction, diuretics, beta-blockers, angiotensin receptor blockers, and bromocriptine. Due to persistent hypotension, dopamine infusion was initiated.

Despite medical therapy, her symptoms persisted, and she was referred to the national cardiology center. Further workup, including computed tomography (CT) and cardiac magnetic resonance imaging, excluded pulmonary embolism, aortic dissection, and myocarditis, confirming the diagnosis of PPCM.

Her clinical course deteriorated, with episodes of ventricular arrhythmias and an inability to wean from inotropic support. The heart team opted for transfer to the cardiac surgery center for evaluation for MCS, given the unavailability of a suitable donor.

Six months postpartum, the patient underwent LVAD implantation as BTT therapy. Postoperatively, she was successfully weaned off inotropic support, with complete resolution of HF symptoms. Her recovery was uneventful, and she was discharged in stable condition.

The patient lived with the LVAD for five years, managing the device and its associated lifestyle adjustments, until a suitable donor heart became available when she was 30 years old. The donor heart was size-matched and compatible with respect to ABO blood type and human leukocyte antigens antibodies.

Heart transplantation with LVAD explantation was done according to modern standards in surgical practice (Figure 1).

Intraoperative transesophageal echocardiography was employed to monitor for intracardiac air, assess biventricular function, valvular competence, and anastomotic integrity. Due to extensive pericardial resection during LVAD explantation, bilateral pericardial reconstruction was performed with bovine pericardial patches (Figure 2).

The total ischemic time of the heart was 140 min. The patient was weaned from cardiopulmonary bypass upon achieving hemodynamic stability and transferred to the intensive care unit.

Serial endomyocardial biopsies confirmed the absence of rejection. The patient was discharged in stable clinical, hemodynamic, and immunobiological condition after four weeks.

Ethics: Written informed consent was obtained from the patient to publish this case report.

DISCUSSION

HF is a complex syndrome characterized by the heart's inability to maintain adequate circulatory output. PPCM is a unique form of HF occurring during late pregnancy or early postpartum, with hallmark LV systolic dysfunction [8, 9].

Medical management remains the cornerstone of PPCM treatment. The BOARD regimen (bromocriptine, oral HF therapies, anticoagulants, vaso-relaxing agents, and diuretics) is commonly employed, with bromocriptine playing a pivotal role by inhibiting prolactin secretion [10].

Reported outcomes in PPCM vary. Up to 72% of patients may experience LV functional recovery (EF > 50%) within 12 months, though some advance to HF or sudden cardiac death [11, 12, 13]. Poor prognostic indicators include LVEF < 30%, LV end-diastolic diameter > 60 mm, and delayed postpartum diagnosis – all present in our patient [14].

If a patient does not respond to maximal medical therapy, surgical intervention is warranted. Up to 10% of PPCM patients may ultimately require heart transplantation [11]. Given the ongoing mismatch between donor heart supply and demand, LVADs have emerged as critical tools in BTT strategies.

LVADs significantly improve survival and quality of life in patients awaiting transplantation [15]. Third-generation devices, such as the HeartMate 3 (Abbott, Chicago, IL, USA), are continuous-flow centrifugal pumps with enhanced durability, lower thrombosis and hemolysis rates, and improved patient outcomes [16–19].

Nonetheless, LVAD presence introduces technical challenges for subsequent cardiac surgery. Reoperative cardiac surgery carries elevated risks of morbidity and mortality,

and the surgical approach must be meticulously planned, including preoperative CT imaging to evaluate LVAD positioning and outflow graft orientation. To minimize ischemic time, transplantation procedures in LVAD recipients require early initiation and coordinated timing between procurement and implantation teams [20, 21, 22].

The bicaval technique offers superior right atrial morphology preservation, facilitates physiological atrial conduction, and reduces supraventricular arrhythmia incidence [23].

Post-transplantation care focuses on optimizing graft function, initiating immunosuppression, monitoring for rejection, and preventing infections [24]. Endomyocardial biopsy remains the gold standard for assessing graft rejection [25].

Although surgical therapy is not the primary approach for PPCM, it remains an essential option for patients with refractory or advanced disease. This case highlights that, despite a prolonged and challenging treatment course, successful heart transplantation is achievable with comprehensive multidisciplinary management.

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REFERENCES

1. Singh A, Irfan H, Ali T, Mughal S, Shaukat A, Jawwad M, et al. Precision medicine in peripartum cardiomyopathy: advancing diagnosis and management through genomic and phenotypic integration. Ann Med Surg (Lond). 2024;86(8):4664–7. [DOI: 10.1097/MS9.0000000000002329] [PMID: 39118717]
2. Michimata H, Sumi T, Nagayama D, Koshino Y, Watanabe H, Yamada Y, et al. Severe heart failure due to peripartum cardiomyopathy. Respirol Case Rep. 2023;11(5):e01137. [DOI: 10.1002/rcr2.1137] [PMID: 37051303]
3. Arany Z. Peripartum Cardiomyopathy. N Engl J Med. 2024;390(2): 154–64. [DOI: 10.1056/NEJMra2306667] [PMID: 38197818]
4. Jawad K, Koziarz A, Dieterlen MT, Garbade J, Etz CD, Saeed D, et al. Long-Term Follow-Up of Mechanical Circulatory Support in Peripartum Cardiomyopathy (PPCM) Refractory to Medical Management: A Multicenter Study. Life (Basel). 2022;12(1):87. [DOI: 10.3390/life12010087] [PMID: 35054480]
5. Goldstein DJ, Naka Y, Horstmanshof D, Ravichandran AK, Schroder J, Ransom J, et al. Association of Clinical Outcomes With Left Ventricular Assist Device Use by Bridge to Transplant or Destination Therapy Intent: The Multicenter Study of MagLev Technology in Patients Undergoing Mechanical Circulatory Support Therapy With HeartMate 3 (MOMENTUM 3) Randomized Clinical Trial. JAMA Cardiol. 2020;5(4):411–9. [DOI: 10.1001/jamacardio.2019.5323] [PMID: 31939996]
6. Antonopoulos M, Bonios MJ, Dimopoulos S, Leontiadis E, Gouziouta A, Kogerakis N, et al. Advanced Heart Failure: Therapeutic Options and Challenges in the Evolving Field of Left Ventricular Assist Devices. J Cardiovasc Dev Dis. 2024;11(2):61. [DOI: 10.3390/jcdd11020061] [PMID: 38392275]
7. Putnik S, Terzic D, Nestorovic E, Markovic D, Ristic M. Successful surgical treatment of terminal heart failure in an adolescent - left ventricular assist device implantation and subsequent heart transplantation. Srp Arh Celok Lek. 2019;147(1–2):78–80. [DOI: 10.2298/sarh170223155p]
8. Melchiorre K, Sharma R, Thilaganathan B. Cardiovascular implications in preeclampsia: an overview. Circulation. 2014;130(8):703–14. [DOI: 10.1161/CIRCULATIONAHA.113.003664] [PMID: 25135127]
9. Bauersachs J, König T, van der Meer P, Petrie MC, Hilfiker-Kleiner D, Mbakwem A, et al. Pathophysiology, diagnosis and management of peripartum cardiomyopathy: a position statement from the Heart Failure Association of the European Society of Cardiology Study Group on peripartum cardiomyopathy. Eur J Heart Fail. 2019;21(7):827–43. [DOI: 10.1002/ejhf.1493] [PMID: 31243866]
10. Arrigo M, Blet A, Mebazaa A. Bromocriptine for the treatment of peripartum cardiomyopathy: welcome on BOARD. Eur Heart J. 2017;38(35):2680–2. [DOI: 10.1093/eurheartj/ehx428] [PMID: 28934838]
11. McNamara DM, Elkayam U, Alharethi R, Damp J, Hsich E, Ewald G, et al. Clinical Outcomes for Peripartum Cardiomyopathy in North America: Results of the IPAC Study (Investigations of Pregnancy-Associated Cardiomyopathy). J Am Coll Cardiol. 2015;66(8):905–14. [DOI: 10.1016/j.jacc.2015.06.1309] [PMID: 26293760]
12. Elkayam U, Tummala PP, Rao K, Akhter MW, Karaalp IS, Wani OR, et al. Maternal and fetal outcomes of subsequent pregnancies in women with peripartum cardiomyopathy. N Engl J Med. 2001;344(21):1567–71. [DOI: 10.1056/NEJM200105243442101] Erratum in: N Engl J Med 2001;345(7):552. [PMID: 11372007]
13. Barone-Rochette G, Rodière M, Lantuejoul S. Value of cardiac MRI in peripartum cardiomyopathy. Arch Cardiovasc Dis. 2011;104(4):263–4. [DOI: 10.1016/j.acvd.2010.07.006] [PMID: 21624795]
14. Honigberg MC, Givertz MM. Peripartum cardiomyopathy. BMJ. 2019;364:k5287. [DOI: 10.1136/bmj.k5287] [PMID: 30700415]
15. Mikic A, Nestorovic E, Bilbija I, Terzic D, Putnik S. Left ventricular assist device implantation and concomitant aortic valve replacement. Srp Arh Celok Lek. 2019;147(11–12):758–61. [DOI: 10.2298/sarh180614066m]
16. Vaidya Y, Riaz S, Dhamoon AS. Left Ventricular Assist Devices. 2023. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. [PMID: 29763016]
17. Suarez-Pierre A, Zhou X, Fraser CD 3rd, Grimm JC, Crawford TC, Lui C, et al. Survival and Functional Status After Bridge-to-Transplant with a Left Ventricular Assist Device. ASAIO J. 2019;65(7):661–7. [DOI: 10.1097/MAT.0000000000000874] [PMID: 30281540]
18. Ripoll JG, Orjuela RB, Ortoleva J, Nabzdyk CS, Dasani S, Bhowmik S, et al. HeartMate 3: Analysis of Outcomes and Future Directions. J Cardiothorac Vasc Anesth. 2024;38(12):3224–33. [DOI: 10.1053/j.jvca.2024.08.016] [PMID: 39214797]
19. Nestorovic E, Terzic D, Putnik S, Ristic A, Ristic M. HeartMate 3 fully magnetically levitated left ventricular assist device for advanced heart failure: Initial Serbian experience. Srp Arh Celok Lek. 2018;146(9–10):584–7. [DOI: 10.2298/sarh170719202n]
20. Tarola C, Fremes S. Commentary: Redo cardiac surgery: Striving for the best but prepared for the worst. J Thorac Cardiovasc Surg. 2022;164(6):1767–8. [DOI: 10.1016/j.jtcvs.2021.01.068] [PMID: 33674064]
21. Garbade J, Meyer AL. Cardiac Transplantation After HeartMate 3 Implantation Inclusive Implantation Tips: Step by Step. Operative Techniques in Thoracic and Cardiovascular Surgery. 2019;24(3):187–203. [DOI: 10.1053/j.optechstcvs.2019.09.001]
22. Cheng A, Slaughter MS. Heart transplantation. J Thorac Dis. 2014;6(8):1105–9. [DOI: 10.3978/j.issn.2072-1439.2014.07.37] [PMID: 25132977]
23. Weiss ES, Nwakanma LU, Russell SB, Conte JV, Shah AS. Outcomes in bicaval versus biatrial techniques in heart transplantation: an analysis of the UNOS database. J Heart Lung Transplant. 2008;27(2):178–83. [DOI: 10.1016/j.healun.2007.11.003] [PMID: 18267224]
24. Hwang NC, Sivathasan C. Review of Postoperative Care for Heart Transplant Recipients. J Cardiothorac Vasc Anesth. 2023;37(1):112–26. [DOI: 10.1053/j.jvca.2022.09.083] [PMID: 36323595]
25. Sinphurmsukskul S, Ariyachaipanich A, Siwamogsatham S, Thammanatsakul K, Puwanant S, Benjacholamas V, et al. Endomyocardial Biopsy and Prevalence of Acute Cellular Rejection in Heart Transplantation. Transplant Proc. 2021;53(1):318–23. [DOI: 10.1016/j.transproceed.2020.08.014] [PMID: 33041079]

Успешна трансплантација срца након дуготрајне потпоре уређајем за леву срчану комору код болеснице са перипарталном кардиомиопатијом – први случај у Србији

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САЖЕТАК

Увод Перипартална кардиомиопатија представља редак и потенцијално животно угрожавајући узрок срчане инсуфицијенције, који се јавља у касној трудноћи или у раном постпорођајном периоду. Иако је медикаментозна терапија терапија првог избора, у случајевима рефракторне или терминалне срчане инсуфицијенције неопходна је хируршка интервенција, укључујући механичку циркулаторну потпору или трансплантацију срца.

Приказ болесника Приказујемо први случај у Србији младе жене са перипарталном кардиомиопатијом, која је успешно лечена уградњом уређаја за механичку потпору функције леве коморе, након чега је изведена ортотопијска трансплантација срца. Болесница, стара 25 година, примљена је са тешком бивентрикуларном срчаном инсуфицијенцијом три и по месеца након порођаја. Упркос оптималној медикаментозној терапији, стање јој се погоршавало, што је захтевало уградњу уређаја за механичку потпору функције леве коморе као моста до трансплантације. На овом уређају провела је пет година, одржавајући одличан квалитет живота, све до појаве одговарајућег донорског срца. Трансплантација срца изведена је техником бикавалне анастомозе, без периоперативних компликација и знакова раног одбацивања графта. Болесница је отпуштена из болнице у стабилном стању.

Закључак Овај случај показује изводљивост и успешност мултидисциплинарног приступа који комбинује уређај за механичку потпору функције леве коморе и трансплантацију срца у лечењу узнапредовале перипарталне кардиомиопатије. Правовремено препознавање прогресије болести и индивидуализовано хируршко планирање од кључног су значаја за постизање повољних исхода у овој високоризичној популацији.

Кључне речи: перипартална кардиомиопатија; уређај за механичку потпору функције леве коморе; трансплантација срца; механичка циркулаторна потпора; мост до трансплантације

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Isolated trans-hiatal colonic herniation causing gastric outlet obstruction

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SUMMARY

Introduction There are four types of hiatus hernia, with type IV being the rarest and occurring in less than 5%. A hiatus hernia that contains only the transverse colon without the stomach and that did not arise because of a traumatic diaphragmatic defect, such as in this case is very seldom, and eight similar cases have been described in the literature so far.

Case outline A 66-year-old man presented to the emergency room with a complaint of nausea and frequent vomiting. Upon examination, the paraumbilical region was profoundly tender. Abdominal X-ray revealed a distended stomach with air-liquid levels in the right upper quadrant. Ultrasound of the abdomen showed a distended, hypotonic stomach and a suspicious solitary mass of the right kidney. Contrast enhanced computed tomography examination showed an isolated herniation of the transverse colon with its respective vascular pedicle, causing consecutive compression and obstruction of the pylorus and duodenal bulb. An open laparotomy was performed – including repositioning of the transverse colon with omentum along with repair of the crural defect and Toupet's fundoplication. In the same act, a right-sided radical nephrectomy was performed. The patient was discharged after 10 days without complications.

Conclusion Isolated trans-hiatal herniation of the colon presents with non-specific symptoms and in the case of acute gastric obstruction is an indication for urgent surgery. Correct diagnosis confirmed by computed tomography and adequate treatment can prevent possible complications.

Keywords: hiatal hernia; paraoesophageal colonic herniation; gastric outlet obstruction

INTRODUCTION

Hiatus hernia represents the migration of intra-abdominally positioned organs intrathoracic into the mediastinum through the esophageal hiatus. There are four types of hiatus hernia (I–IV), with type IV being the rarest and occurring in less than 5% [1–4]. A hiatus hernia containing only part of the transverse colon, without the stomach, which did not occur because of a traumatic diaphragmatic defect, as in this case, is very rare, and so far, eight similar cases have been described in the literature [5–12]. Based on the mentioned cases, the potential modification of the existing classification is questioned, where type IVa could correspond to isolated herniation of abdominal organs without the stomach [13].

This patient experienced an isolated colonic herniation and incarceration treated by Toupet's fundoplication [14, 15].

CASE REPORT

A 66-year-old man presented to the emergency room with nausea and frequent vomiting over the course of the last ten days, with no significant medical history, trauma, or previous operations. Upon physical examination, the epigastric region was profoundly tender. Initial laboratory findings showed a white blood cell count of 9×10^9/l, C-reactive protein of 100 mg/dl. Abdominal X-ray showed a distended stomach with an air fluid level in the right upper quadrant. Ultrasound of the abdomen revealed a distended, hypotonic stomach and a suspicious tumorous formation of the right kidney. Contrast enhanced computed tomography examination showed a paraoesophageal herniation of the transverse colon with its mesocolon and respective vascular pedicle, propagating through the 150 millimeters wide hiatus cranially and forming an angulation. The herniated colon segment was causing direct consequential compression and obstruction of the pylorus and duodenal bulb, while the stomach was positioned intra-abdominally, with no torsion and distended with fluid contents. The vascular structures of the colon were not showing sign of obstruction, revealing normal postcontrast enhancement (Figures 1 and 2).

There was an expansive formation on the lower pole of the right kidney, presenting with radiological features of renocellular carcinoma. The patient was admitted to the surgical department where nasogastric decompression was performed, evacuating over 3000 milliliters of stomach contents. An open laparotomy was performed, and intraoperative findings confirmed that the stomach was distended and positioned intra-abdominally, with its outlet compressed by the colonic herniation, edematous stomach serosa and surrounding adipose tissue, and a significantly thinned right diaphragmatic crus. The transverse colon with omentum was then

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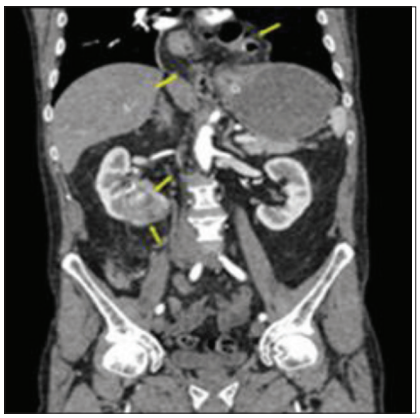

Figure 1. Coronal computed tomography image showing isolated intrathoracic colonic herniation (yellow arrows) and a solitary mass in the right kidney

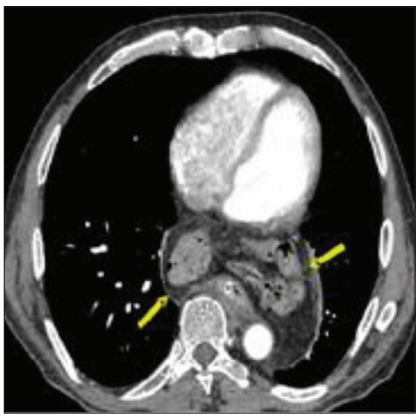

Figure 2. Axial computed tomography image showing isolated intrathoracic colonic herniation (yellow arrows)

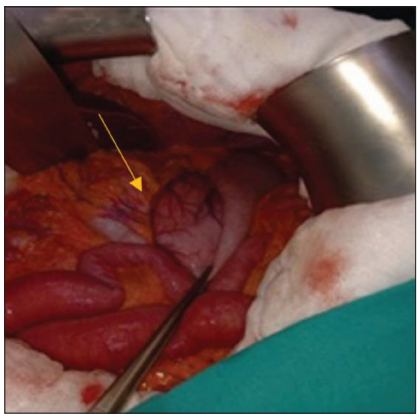

Figure 3. Intraoperative findings – isolated intrathoracic colonic herniation (yellow arrow)

repositioned, the crural defect repaired and Toupet's fundoplication was performed. In the same act a right-sided radical nephrectomy was executed (Figure 3). Following an uneventful postoperative course, the patient was discharged after 10 days. Since then, the patient has been enrolled in a routine follow-up program and reports feeling well.

Ethics: Written consent to publish all shown material was obtained from the patient.

DISCUSSION

Limited number of cases prevents from drawing any general conclusion.

This condition can present with a wide array of clinical manifestations, ranging from minimal epigastric discomfort to dyspnea, dysphagia, nausea, and vomiting, even chest and epigastric pain [16].

In this case, we identified no significant risk factors associated with elevated intra-abdominal pressure, concluding that the most probable etiology was a congenital defect with self-reducing hiatus hernia that progressed to subsequent irreducible colonic migration presenting with gastric outlet obstruction [17, 18, 19].

This direct compression of the gastric outlet caused the patient to present with nausea and vomiting. Intraoperative findings were conclusive with chronic hiatal herniation, showing the gastric outlet displaced upwards and compressed by the herniated transverse colon and mesocolon, edematous stomach serosa and surrounding adipose tissue, and a significantly thinned right diaphragmatic crus. Concerning this case report, there was no endoscopy performed before or during this hospitalization, as there were no specific symptoms, and no evidence of preexisting gastric hiatal herniation.

The natural course of hiatal hernia can become complicated by volvulus, incarceration, perforation, or aspiration pneumonia.

In literature review, there are eight other documented cases of isolated trans-hiatal colonic herniation in absence of intrathoracic stomach. Three patients of these were successfully treated by laparoscopic repair, and the rest, including our case, underwent operative repair [3, 5, 6].

The only other documented case of isolated colonic hiatal herniation presenting with gastric outlet obstruction was delivered by Self and Munro [6].

Considering different clinical manifestations, contrast enhanced CT is indispensable in establishing the correct diagnosis. Even with a limited number of cases including isolated intrathoracic colonic migration in literature, the question arises about a potential revision of the existing classification of hiatal hernia types, where isolated trans-hiatal colonic hernia could be classified into a new subtype IVa [13].

Conflict of interests: None declared.

REFERENCES

1. Kim P, Turcotte J, Park A. Hiatal hernia classification – Way past its shelf life. Surgery. 2021;170(2):642–3. [DOI: 10.1016/j.surg.2021.02.062] [PMID: 33867168]
2. Migaczewski M, Grzesiak-Kuik A, Pędziwiatr M, Budzyński A. Laparoscopic treatment of type III and IV hiatal hernia - authors' experience. Wideochir Inne Tech Maloinwazyjne. 2014;9(2):157–63. [DOI: 10.5114/wiitm.2014.41625] [PMID: 25097681]
3. Smith RE, Sharma S, Shahjehan RD. Hiatal Hernia. 2024. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. [PMID: 32965871]
4. Krause W, Roberts J, Garcia-Montilla RJ. Bowel in Chest: Type IV Hiatal Hernia. Clinical Medicine & Research. 2016;14(2): 93–6. [DOI: 10.3121/cmr.2016.1332] [PMID: 27401794]
5. Altinkaya N, Koc Z, Alkan O, Senay D. MDCT diagnosis of isolated colonic hernia through the esophageal hiatus. Balkan Medical Journal 2010;28:111–2. [DOI: 10.5174/tutfd.2009.02082.1]
6. Self D, Munro W. Isolated colonic hernia through the oesophageal hiatus causing gastric outlet obstruction. ANZ J Surg. 2019;89(7–8):E352–E353. [DOI: 10.1111/ans.14481] [PMID: 29573112]
7. Tabira Y, Yoshida Y, Sakaguchi T, Yoshimatsu S. Isolated colonic hernia through the esophageal hiatus. Dis Esophagus. 2005;18(4):283–6. [DOI: 10.1111/j.1442-2050.2005.00498] [PMID: 16128788]
8. Felsher J, Brodsky J, Brody F. Isolated trans-hiatal colonic herniation. J Laparoendosc Adv Surg Tech A. 2003;13(2):105–8. [DOI: 10.1089/109264203764654731] [PMID: 12737724]

9. Ooi K, Berney C. Laparoscopic repair of gastric volvulus secondary to transverse colon diaphragmatic hernia. Med J Aust. 2008;189(5):294–5. [DOI: 10.5694/j.1326-5377.2008.tb02036.x] [PMID: 18759733]
10. Itabashi Y, Baba T, Kato S, Sasaki M. A case of esophageal hiatal hernia with incarcerated transverse colon. Jpn J Gastroenterol Surg. 2004;37(5):479–82. [DOI: 10.5833/jjgs.37.479]
11. Yildiz SY, Berkem H, Yuksel BC, Ozel H, Hengirmen S. Isolated intrathoracic hiatal herniation of the twisted sigmoid colon: report of a case. Dis Colon Rectum. 2009;52(4):740–1. [DOI: 10.1007/DCR.0b013e318199dbe6] [PMID: 19404083]
12. Plewka M, Peruga J, Chrzanowski L. Isolated Intrathoracic Hiatal Colonic Hernia Mimicking Acute Coronary Syndrome – a Case report. Polski Przeglad Kardiologiczny. 2010;12(1):161–3.
13. Maeda S, Ito S, Hosoda K. Isolated esophageal hiatal hernia of the colon: A case report and review of literature. Asian J Endosc Surg. 2025;18(1):e13400. [DOI: 10.1111/ases.13400] [PMID: 39477344]
14. Singhal VK, Suleman AM, NufraSenofer, Singhal VV. Current Trends in the Management of Hiatal Hernia: A Literature Review of 10 Years of Data. Cureus. 2024;16(10):e7192. [DOI: 10.7759/cureus.71921] [PMID: 39564064]
15. Rashid F, Thangarajah T, Mulvey D, Larvin M, Iftikhar SY. A review article on gastric volvulus: a challenge to diagnosis and management. Int J Surg. 2010;8(1):18–24. [DOI: 10.1016/j.ijsu.2009.11.002] [PMID: 19900595]
16. Abu-Freha N, Guterman R, Elhayany R, Yitzhak A, Hudes SS, Fich A. Hiatal hernia: risk factors, and clinical and endoscopic aspects in gastroscopy. Gastroenterol Rep (Oxf). 2024;12:goae086. [DOI: 10.1093/gastro/goae086] [PMID: 39281268]
17. Albasheer O, Hakami N, Ahmed AA. Giant Morgagni hernia with transthoracic herniation of the left liver lobe and transverse colon: a case report. J Med Case Rep. 2023;17(1):165. [DOI: 10.1186/s13256-023-03914-0] [PMID: 37088823]
18. Nugraha HG, Agustina M, Nataprawira HM. Diagnostic challenges of hiatal hernia Type IV: An imaging perspective. Radiol Case Rep. 2024;20(1):437–41. [DOI: 10.1016/j.radcr.2024.09.147] [PMID: 39534747]
19. Fuchs KH, Kafetzis I, Hann A, Meining A. Hiatal Hernias Revisited-A Systematic Review of Definitions, Classifications, and Applications. Life (Basel). 2024;14(9):1145. [DOI: 10.3390/life14091145] [PMID: 39337928]

Изолована трансхијатална хернијација трансверзалног колона као редак узрок опструкције желуца

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САЖЕТАК

Увод Хијатус хернија представља миграцију интраабдоминално позиционираних органа интраторакално у медијастинум кроз езофагеални хијатус. Постоје четири типа хијатус хернije (I–IV), при чему је тип IV најређи и јавља се у мање од 5% случајева. Хијатус хернија која садржи само део трансверзалног колона без желуца и која није настала као резултат трауматског дефекта дијафрагме, као у нашем случају, веома је ретка и до сада је описано осам сличних случајева у литератури. На основу наведених случајева, доводи се у питање могућа допуна постојеће класификације, где би тип IVa могао одговарати изолованој хернијацији абдоминалних органа без присуства желуца.

Приказ случаја Мушкарац старости 66 година јавио се у хитну службу жалећи се на мучнину и учестало повраћање. Нативна радиографија абдомена указивала је на дистендиран желудац са аероликвидним нивоом у десном горњем квадранту. Ултразвучни преглед показао је дистендиран, хипотоничан желудац и туморску формацију десног бубрега. Компјутеризована томографија са интравенским контрастом показала је параезофагеалну хернијацију трансверзалног колона са припадајућом васкуларном петељком и са последичном компресијом и опструкцијом пилоруса и булбуса дуоденума. Урађена је хитна лапаротомија са репозиционирањем трансверзалног колона са оментумом, крурални дефект је репариран, извршена је фундопликација по Тупету и десна радикална нефректомија. Постоперативни ток је протекао уредно и болесник је отпуштен након десет дана.

Закључак Изолована трансхијатална хернијација колона испољава се неспецифичним симптомима и, у случају акутне опструкције желуца, представља индикацију за хитну операцију. Прецизна дијагноза потврђена компјутеризованом томографијом и адекватан третман могу спречити могуће компликације.

Кључне речи: хијатус хернија; параезофагеална хернијација колона; гастроинтестинална опструкција

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Migrated bullet in the bladder presenting 30 years after a gunshot wound to the gluteal region

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SUMMARY

Introduction In current practice, genitourinary trauma secondary to gunshot wounds is relatively rare. Even less common is the migration of a bullet decades after the trauma, with only a few cases described in the literature.

This article illustrates the sporadic occurrence of bullet migration into the urinary system. It underscores the importance of prompt diagnosis and timely treatment, as the time period from the trauma occurrence to symptom onset can be extremely long.

Case outline We present a case of a retained bullet that migrated into the bladder 30 years after the injury in the gluteal region. This is the longest period from the occurrence of the gunshot wound to the onset of symptoms described in the literature. After failed attempts at retrieving the bullet endoscopically, a small cystostomy was performed with successful evacuation of the foreign body.

Conclusion The time gap from the occurrence of the trauma to the appearance of symptoms can complicate the diagnostic and treatment process. The history of a gunshot wound in this region suggests the exclusion of bullet or shrapnel migration into the bladder, regardless of the time distance since the trauma. Although migration is rare, it highlights the need for both short-term and long-term follow-up of patients with retained bullets and shrapnel.

Keywords: foreign-body migration; complications; urogenital system injuries; urogenital system surgery

INTRODUCTION

Foreign-body entry into the urinary tract is most often due to the insertion of objects into the urethra by the patient or an intimate partner, and less often to ballistic trauma with direct involvement of the urinary tract. Gunshot injuries to the genitourinary system are rare in daily practice in developed countries. The genitourinary system is affected in about 10.5% of gunshot wounds [1]. The bladder is the second most commonly affected part after the kidney [2]. When considering injuries to the lower urinary tract, bladder involvement accounts for 37.93% of them [3]. Much less commonly, a foreign body can migrate into the bladder from the perivesical tissues after trauma or surgery. Given the scarcity of cases in the literature, attention should be paid to the possibility of late migration of a foreign body into the urinary system, as it may complicate and delay the diagnostic process and treatment.

CASE REPORT

A 63-year-old man presented to the urology office with an acute onset of dysuria, pollakiuria, difficult urination with cessation of the urinary stream acting like a valve mechanism, and pain in the suprapubic area. The complaints started suddenly a few weeks before the visit. The patient also reported a feeling of not emptying the bladder and several episodes of blood in the urine after physical exertion. He denied a history of urinary stones. The antibiotic therapy

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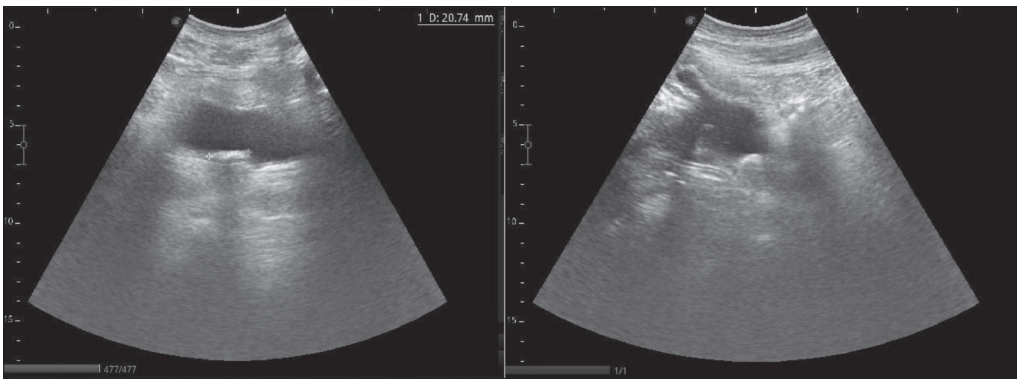

Figure 1. Ultrasound image of the bladder with hyperechoic structure inside

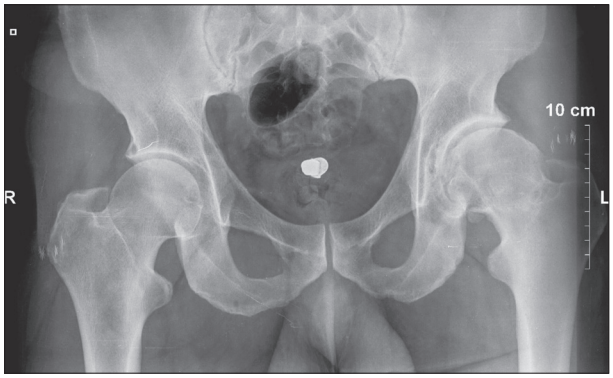

Figure 2. X-ray of the pelvis showing the foreign body

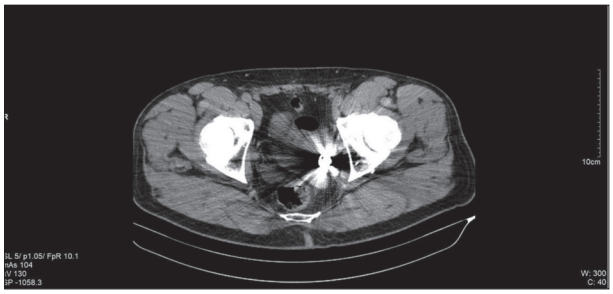

Figure 3. Computed tomography of the pelvis showing a foreign body with metallic density in the bladder cavity

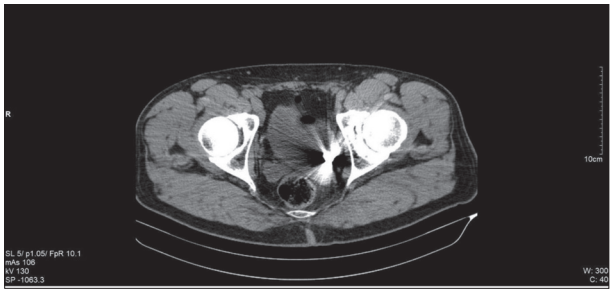

Figure 4. Computer tomography of the pelvis showing a foreign body with metallic density in the bladder cavity

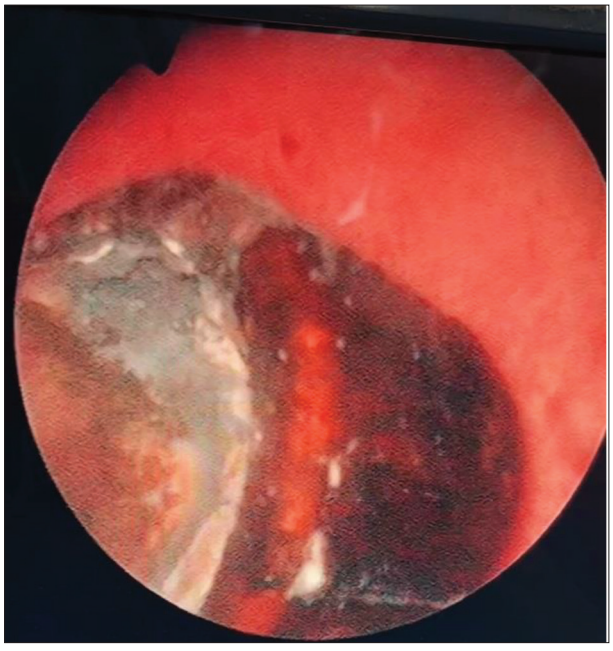

Figure 5. Endoscopic view of the bullet that migrated into the bladder

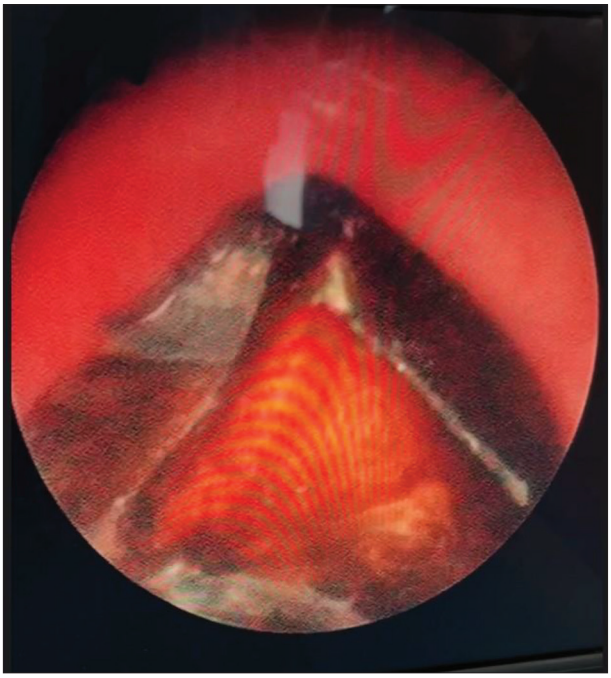

Figure 6. Endoscopic view of the bullet that migrated into the bladder

prescribed by his personal physician had no effect, after which he was referred to a urologist. After a thorough history of previous illnesses and hospitalizations, he reported a gunshot wound in the gluteal region 30 years previously – during treatment of the wound, part of the bullet had been evacuated, but a fragment had been left in the wound channel. The wound healed without complications.

An ultrasound examination was performed, which revealed a hyperechoic shadow within the bladder measuring about 20 mm (Figure 1). An X-ray of the kidney–ureter–bladder region was performed, showing a metal-density shadow about 16 mm in diameter (Figure 2). Computed tomography of the abdomen and pelvis showed a metallic-density foreign body, suspicious for part of a bullet, measuring about 14 mm, with an intact bladder wall and no extravasation of contrast material. The kidneys were without hydronephrosis (Figures 3 and 4).

Cystoscopy confirmed a migrated, free-floating bullet fragment in the bladder (Figures 5 and 6). On careful examination, no defect or fistula was found on the bladder wall. An attempt was made to remove the foreign body endoscopically, but it did not pass through the bladder neck (Figures 7 and 8).

The possibility of retrograde ejaculation after a bladder-neck incision and the risk of urethral injury, given the size of the foreign body, were explained to the patient. An informed decision was made to perform a cystotomy with foreign-body extraction. A suprapubic incision with a small cystotomy was performed, and a part of the bullet measuring 14 × 10 mm was removed (Figures 9 and 10).

Under direct inspection, the bladder wall was found to be intact. The bladder was closed in two layers, and a catheter was placed for five days. The postoperative period went without complication*; the* patient was discharged five days after the surgery with complete resolution of complaints. He was followed up for a period of six months,

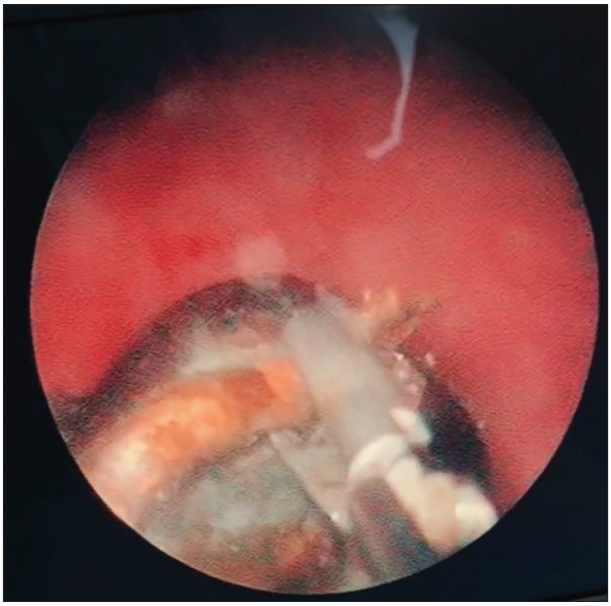

Figure 7. Unsuccessful endoscopic attempt to remove the bullet from the bladder

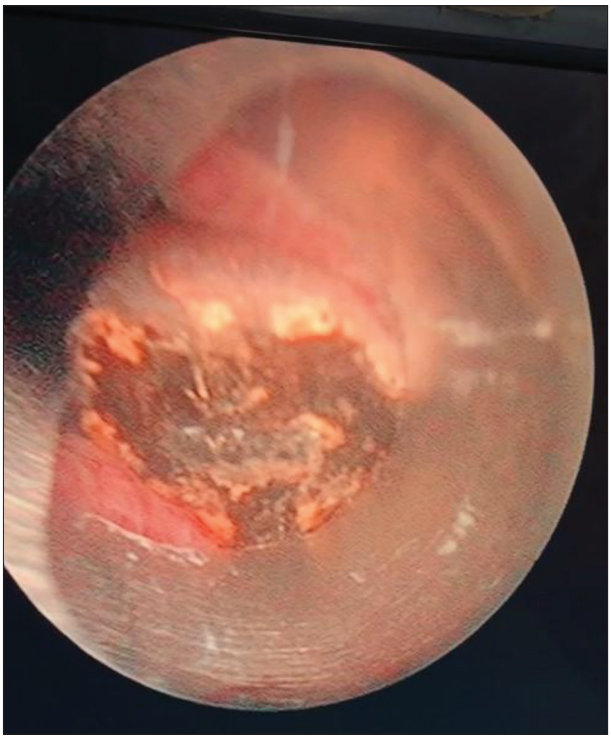

Figure 8. Unsuccessful endoscopic attempt to remove the bullet from the bladder

without any complaints from the genitourinary system. The postoperative ultrasound found no abnormalities.

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

DISCUSSION

We present one of the few cases in the literature of a bullet migrating into the bladder several decades after the

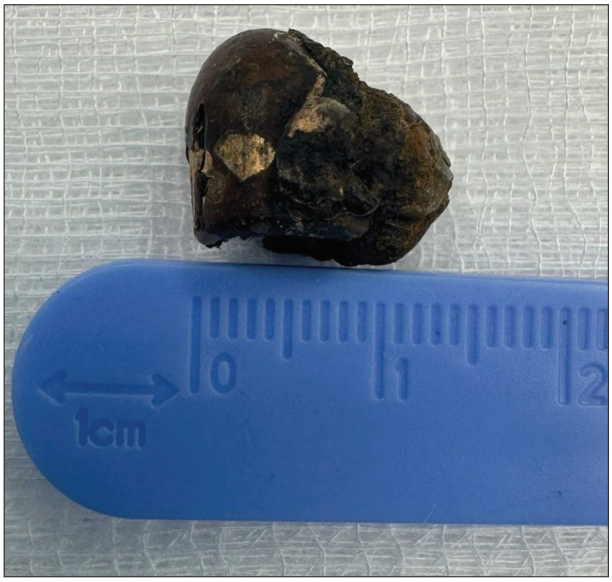

Figure 9. The extracted bullet

Figure 10. The extracted bullet

gunshot wound. To our knowledge, this is the longest period from injury to bullet migration and onset of complaints reported in the literature. The sudden onset of complaints and the gunshot-wound scar in the gluteal region point to a projectile migrating 30 years after the trauma. Another fact confirming the recent migration of the bullet is the absence of incrustations on the metal surface. In similar cases in the literature, at the onset of the complaints, encrustations have already formed around the bullet or the shrapnel fragment, leading to laser lithotripsy of the encrusted shell at the first stage [4].

Primary bullet penetration into the bladder following a gunshot wound often presents with hematuria [5]. The possibility of involvement of other parts of the genitourinary tract due to their proximity should not be forgotten. Injury to them can occur both during trauma and during migration of the foreign body [6]. Bullet migration into the bladder shortly after extraperitoneal trauma has been

described, with no contrast extravasation identified on imaging studies either during the extravesical placement of the bullet or after migration into the bladder several days later [7].

The migration of a bullet in the urinary tract is most often manifested clinically with dysuric symptoms and hematuria. A review of the literature shows that it can also occur with the onset of acute urinary retention after causing obstruction by entering the urethra [8]. Migration of the foreign body into the ureter can also manifest as renal colic [9].

The variety of complaints and the different size of the migrated foreign body determine the subsequent behavior, which can be both conservative and invasive. Conservative management is appropriate in cases where the size and location of the bullet allow for its spontaneous elimination. Consideration should include the use of alpha-blockers and non-steroidal anti-inflammatory drugs. When, despite conservative measures, the foreign body cannot be eliminated, a more invasive approach should be undertaken. Depending on the location and size, this varies from endoscopic extraction of the foreign body [5] to surgical removal by cystotomy – which was the approach in our case.

In conclusion, only a small number of cases of bullet migration into the bladder have been described. After consulting the literature, the 30-year period from the occurrence of the gunshot injury to the onset of symptoms presented by us is the longest described so far. The time gap from the occurrence of the trauma to the appearance of symptoms can complicate the diagnostic and treatment process. When there is a sudden onset of dysuric complaints, hematuria, and a history of a gunshot wound in this area, the migration of a bullet or shrapnel into the bladder should be ruled out, regardless of the time distance since the trauma. Removal of the bullet in such cases should be considered because of irritative complaints and the possible complications that may occur. Methods of choice that have proven their effectiveness and safety are endoscopic removal and cystotomy.

Conflict of interest: None declared.

REFERENCES

1. Najibi S, Tannast M, Latini JM. Civilian gunshot wounds to the genitourinary tract: incidence, anatomic distribution, associated injuries, and outcomes. Urology. 2010;76(4):977–81; discussion 981. [DOI: 10.1016/j.urology.2010.01.092] [PMID: 20605196]
2. Tejuoso A, George A, Johnson S, Geller AE, Kapple P, Ziegler C, et al. Gunshot wound injury to the genitourinary tract: a 4-year retrospective review at an academic level 1 trauma center. Transl Androl Urol. 2024;13(3):406–13. [DOI: 10.21037/tau-23-466] [PMID: 38590963]
3. Cissé D, Diallo MS, Diakité AS, Traoré A, Koné M, Traoré D, et al. Blessures par arme à feu du bas appareil urinaire en période de crise sécuritaire à l'hôpital Sominé Dolo de Mopti (Mali) : aspects épidémiologiques et diagnostiques [Gunshot wounds of low urinary tract in period of security crisis at the Sominé Dolo Hospital of Mopti (Mali): Epidemiological and diagnostic aspects]. Prog Urol. 2023;33(10):463–8. [DOI: 10.1016/j.purol.2023.07.002] [PMID: 37495441]
4. Marantidis J, Biggs G. Migrated bullet in the bladder presenting 18 years after a gunshot wound. Urol Case Rep. 2019;28:101016. [DOI: 10.1016/j.eucr.2019.101016] [PMID: 31720229]
5. Friedman AA, Trinh QD, Kaul S, Bhandari A. Complete endoscopic management of a retained bullet in the bladder. Can Urol Assoc J. 2013;7(1–2):E143–5. [DOI: 10.5489/cuaj.258] [PMID: 23671506]
6. Tabei SS, Lippold B, Baas W, Murphy G. Penetrating posterior urethral injuries: case report and management strategies. Case Rep Urol. 2024;2024:7839379. [DOI: 10.1155/2024/7839379] [PMID: 39104899]
7. Mehta C, Loecher M, Sih A, Reese AC. A report of a retained bullet in the bladder which migrated from an extraperitoneal injury. Urol Case Rep. 2020;34:101463. [DOI: 10.1016/j.eucr.2020.101463] [PMID: 33224728]
8. Molla YD, Mekonnen DC, Gelaw TB, Sendekie TA. Wandering projectile, a rare cause of acute urinary retention. BMC Urol. 2023;23(1):36. [DOI: 10.1186/s12894-023-01204-x] [PMID: 36899331]
9. Gutvert R, Kobirnichenko A, Bidula Y, Balabanyk V. Successful management of penetrating shrapnel injury to the left ureter with delayed projectile migration through the urinary tract. IJU Case Rep. 2023;6(6):362–4. [DOI: 10.1002/iju5.12623] [PMID: 37928281]

Миграција метка у бешику 30 година након прострелне ране у глутеалној регији

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САЖЕТАК

Увод У досадашњој пракси генитоуринарне трауме настале услед прострелних рана релативно су ретке. Још ређа је миграција метка деценијама након повреде, са неколико случајева описаних у литератури.

Овај чланак илуструје спорадичну појаву миграције метка у уринарни систем и подвлачи значај брзе дијагнозе и благовременог лечења, јер период од повреде до појаве симптома може бити изузетно дуг.

Приказ болесника Представљамо случај задржаног метка који је мигрирао у бешику 30 година након повреде у глутеалној регији. Ово је најдужи период од настанка прострелне ране до појаве симптома описан у литератури. Након неуспелих покушаја ендоскопског извлачења метка, урађена је мала цистостома са успешним уклањањем страног тела.

Закључак Временски размак од настанка трауме до појаве симптома може да отежа процес дијагностике и лечења. Досадашњи извештаји о прострелним ранама у овој регији углавном су искључивали могућност миграције метка или гелера у бешику, без обзира на временску дистанцу од повреде. Иако је миграција ретка, она указује на потребу за краткорочним и дугорочним праћењем болесника са задржаним мецима и гелерима.

Кључне речи: миграција страног тела; компликације; повреде урогениталног система; хирургија урогениталног система

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Anesthesia in an infant with spinal muscular atrophy type 1 for ventriculoperitoneal shunt placement

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SUMMARY
Introduction Spinal muscular atrophy (SMA) is a rare inherited disease in the pediatric population. The most important risk factor associated with anesthesia is increased sensitivity to opioids and neuromuscular blockers as well as problems with airway management and breathing. We present a case report of an eight-month-old female infant undergoing ventriculoperitoneal shunt placement.
Case outline The infant underwent ventriculoperitoneal shunt placement for hydrocephalus following nusinersen intrathecal injection. The premedication with benzodiazepines is omitted. Except for SMA, the child was considered without comorbidity. The general endotracheal anesthesia was managed with inhalational sevoflurane. The opioid analgesics as well as rocuronium for neuromuscular blockade in a single dose were administered to the child. After the surgery, the child was awake in the operating theater.
Conclusion General endotracheal anesthesia in infants with SMA can be safely performed. Intravenous anesthesia as well as inhalational anesthesia with sevoflurane are acceptable possibilities.
Keywords: spinal muscular atrophy; anesthesia; inhalational; ventriculoperitoneal shunt

INTRODUCTION

Spinal muscular atrophy (SMA) type 1 is a rare inherited disease in a pediatric population [1]. It is characterized by muscular hypotonia and delayed motor development [2]. General endotracheal anesthesia is challenging in these children because of potential problems with the airway and breathing as well as increased sensitivity to opioids and neuromuscular blockers [3]. We present a case report of an eight-month-old female infant who underwent ventriculoperitoneal shunt placement.

CASE REPORT

An eight-month-old female infant with the diagnosis of SMA type 1 (Werding–Hoffmann disease) was admitted to the Neurosurgery Clinic of the University Clinical Center of Serbia, in Belgrade for treatment of obstructive hydrocephalus as a consequence of intrathecal administration of nusinersen.

This is the fourth child from the fourth pregnancy which was appropriately monitored and uncomplicated. The child was born in the 39th gestational week, by vaginal delivery. At birth, the child had a body weight of 3820 g and a body length of 54 cm, with a head diameter of 37cm and an Apgar score of 9/10. During pregnancy, the mother has noticed that "the baby was not that active." After birth, except for reduced movements, the parents did not notice anything suspicious in the infant.

During a regular pediatric follow-up, the pediatrician noticed the presence of hypotonia, so the infant was sent to a pediatric neurologist for examination. On the 43rd day of life, the child was admitted to the Pediatric Neurology department for evaluation of severe hypotonia. Because of suspicion of SMA type 1, molecular and genetic consultation is indicated to determine the state of mutation in the *SMN1* gene. Genetic evaluation revealed that there is homozygous deletion of *SMN1* genes and two copies of *SMN2* genes. The diagnosis of SMA type 1 was done. After the confirmed diagnosis of SMA type 1, the indication for nusinersen administration according to protocols was made.

On the 48th day of life, the first dose of nusinersen, 12 mg, was applied intrathecally, and the intervention was done without complications. The first of three doses was administered at 14-day intervals, and the fourth dose was administered 30 days after the third dose. After the fourth dose, the parents noticed an enlarged head circumference, so the magnetic resonance imaging of the cranium was done. It showed the presence of hydrocephalus. The neurosurgeon indicated ventriculoperitoneal shunt placement. The pediatric neurologist indicated interruption of nusinersen intrathecal therapy, but risdiplam in one daily dose of 0.2 mg/kg *per os* was initiated. The infant was transferred to a tertiary referent neurosurgical center.

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On admission, the infant had a body mass weight of nine kilograms and a head circumference of 40 cm with fontanela major 3 × 3 cm in the level of cranium bones. The infant was awake, and interested in the environment, with generalized weakness, preserved swallowing, and cough. She was unable to sit independently and to crawl. The pediatric neurologist indicated a continuation of risdiplam therapy, as well as on the day of surgery. She was examined by a pediatrician, and there were no contraindications for surgery. There were no recommendations for preoperative medical respiratory support, the child was American society of anesthesiologists physical status classification system score 3. In-room air, the oxygen saturation of hemoglobin was 100% (Figure 1).

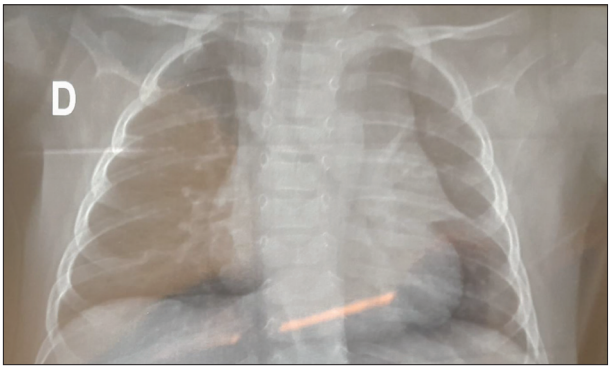

Figure 1. Preoperative chest X-ray in the infant with spinal muscular atrophy type 1

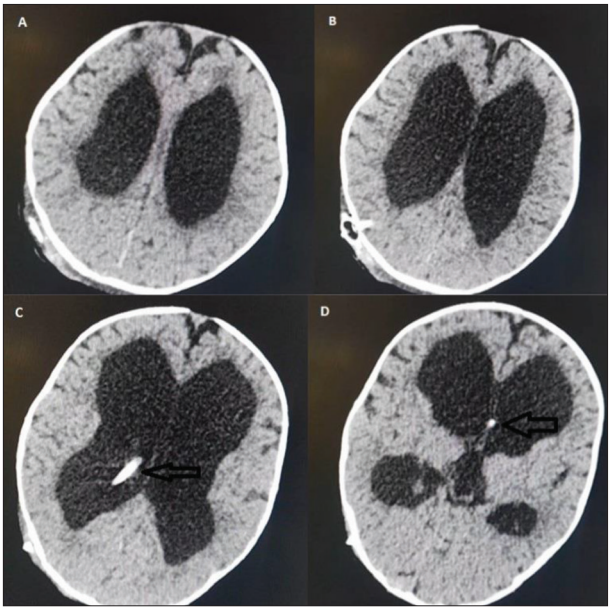

Figure 2. Postoperative native computed tomography of endocranium scan (after ventriculoperitoneal shunt placement): A, B – still dilated lateral ventricles; C – shunt in situ ("arrow"); D – tip of the shunt in left lateral ventricle ("arrow")

Premedication was intentionally omitted due to SMA. Standard monitoring of electrocardiography, non-invasive blood pressure, and pulse oximetry was done. Arterial blood pressure was 125/84 mmHg and heart rate 130/min. The infant was introduced into general endotracheal with O_2/air mixture and sevoflurane at 8 vol% which decreased to 3 vol% till the intubation. After the infant became unresponsive, we started with manual bag ventilation. The nurse inserted and fixed an intravenous cannula. Before intubation, fentanyl 25 mcg and rocuronium 5 mg intravenously were administered and were not repeated. The orotracheal intubation was done with endotracheal tube number 4, with a non-inflated cuff, fixed on 11 cm. The position of the tube was confirmed with chest auscultation and capnography. The throat pack was done. After intubation, mechanical ventilation was initiated (pressure-controlled ventilation with inspiratory pressure of 15 cm H_2O and end-expiratory pressure of 5 cm H_2O, respiratory rate 25/min, FiO_2 50%). End-tidal CO_2 was maintained between 37 and 41 mmHg. Anesthesia was maintained with sevoflurane between 2 and 3 vol% and a total O_2/air flow of 3 L/min. Antibiotic prophylaxis was done with ceftriaxone 500 mg in 100 mL of normal saline infusion.

As usual, the ventriculoperitoneal shunt was placed on the right side: the skin and soft tissue were cut on the parietooccipital part of the scull on the right side (Figure 2). The burr hole was formed. After the cut of the dura mater, the cranial part of the catheter was put into the right lateral ventricle. Clear cerebrospinal fluid occurred in the stream. The catheter was then connected to the pump for medium pressure. The distal portion of the system was positioned in the peritoneum, paraumbilically on the right side. Its function was verified, after which the layers were closed with sutures.

Sevoflurane was stopped and the oxygen was turned to 80% on emergence. The infant was manually ventilated. Neuromuscular blockade was reversed with neostigmine 0.5 mg and atropine 0.2 mg intravenously. After a few minutes, the infant became fully awake and was crying loudly. Extubation was carried out in the inspiratory phase, without endotracheal suction. After extubation, SpO_2 was 99–100% on oxygen flow 4l/min and 97–98% on room air. The infant was transferred to the intensive care unit for further monitoring and oxygenation. Antibiotic therapy with ceftriaxone once a day was continued. Analgesia was provided with acetaminophen 150 mg (15 mL) intravenously every six hours. Intravenous infusion in equal parts of 10% dextrose solution and normal saline, 40 mL/h was administered.

The next day, she was transferred to the neurosurgical ward in good condition – awake, eupneic, acyanotic, and afebrile. The therapy with risdiplam was continued postoperatively. On the second day after surgery, the infant developed inspiratory stridor. After pediatrician examination, the next therapy is administered: methylprednisolone 1 mg/kg intravenously every 12 hours and budesonide inhalations, 0.5 mg diluted with 2 mL of normal saline, every 12 hours. In laboratory findings, C-reactive protein was 11.5 mg/L, and white blood cells were 17.2×10^9/mL. Antibiotic therapy with ceftriaxone was continued. On the fourth day after surgery, the stridor was in resolution, white blood cells were 17.1×10^9/mL, and C-reactive protein was 4.8 mg/mL. On the fifth postoperative day, the infant was discharged from the hospital.

Ethics: We obtained informed consent from the mother to publish the infant case, with total protection of the patient's data.

DISCUSSION

SMA type 1 is a rare inherited recessive genetic condition diagnosed in the first months of life. A homozygous deletion of *SMN1* exon 7 is the most common cause of SMA type 1 and causes the clinical picture of SMA [3]. If untreated, this disease leads to progressive generalized weakness. Affected children usually have hypotonia, difficulty swallowing and cough, which can result in aspiration pneumonia and respiratory failure [2].

Anesthetic care for infants with SMA type 1 presents significant challenges, and literature on optimal strategies remains limited [1–9]. Anesthesia in this population can be safely performed when individualized care is guided by comprehensive risk assessment tools, such as preoperative pulmonary function evaluation (when feasible), swallowing assessments, and multidisciplinary planning. Detailed history regarding respiratory infections, feeding difficulties, and ventilatory support (e.g., use of non-invasive ventilation at home) is essential to stratify perioperative risk.

There is no universally accepted anesthetic regimen for SMA type 1; however, certain principles are broadly recommended. The use of depolarizing neuromuscular blockers such as succinylcholine is contraindicated due to the risk of hyperkalemia, rhabdomyolysis, and cardiac arrest, which is related to the upregulation of extrajunctional acetylcholine receptors [3, 5]. Non-depolarizing neuromuscular blockers, such as rocuronium or cisatracurium, can be used cautiously at reduced doses, ideally guided by neuromuscular monitoring (e.g., train-of-four). However, the reliability of such monitoring in SMA patients is debated due to altered neuromuscular transmission [5]. Some authors advocate avoiding muscle relaxants entirely and relying on deep inhalational or intravenous anesthesia [5, 6].

Regarding analgesia, opioid sensitivity is a concern due to impaired clearance and weakened respiratory drive. Short-acting opioids like remifentanil are preferred intraoperatively because of their rapid metabolism and minimal residual effect [2, 8]. Postoperative pain control can then be managed with multimodal analgesia strategies to minimize opioid requirements.

Both intravenous (e.g., propofol) and inhalational (e.g., sevoflurane) anesthetic agents have been used effectively in this population. There is no clear evidence favoring one method over the other in terms of safety or outcomes [2, 5, 9]. The choice often depends on institutional protocols and the anticipated need for postoperative ventilation.

Respiratory management is a central concern in SMA type 1, both preoperatively and postoperatively. Preoperative preparation should include assessment of airway patency, history of aspiration, and baseline respiratory support. Techniques such as chest physiotherapy, suctioning, and optimization of non-invasive ventilation are beneficial. Some institutions advocate preemptive admission to intensive care and early involvement of respiratory therapists.

Intraoperatively, gentle ventilation strategies should be employed to avoid barotrauma, and extubation decisions must consider the child's ability to maintain airway patency and effective ventilation. Postoperative respiratory complications, such as aspiration, atelectasis, or stridor (as seen in our case), may require escalation to non-invasive or invasive mechanical ventilation. In our case, the development of inspiratory stridor necessitated the use of systemic and inhalational corticosteroids, as well as continued antibiotic therapy.

Importantly, postoperative care should involve close collaboration with pediatricians, pulmonologists, and anesthesiologists, emphasizing the need for a multidisciplinary approach. These children should ideally be treated in tertiary care centers equipped to manage complex airway and neuromuscular issues.

General endotracheal anesthesia can be safely administered to infants with SMA, provided appropriate precautions are taken. Both intravenous and inhalational anesthesia using agents such as sevoflurane are acceptable options. Anesthesia management should include the use of reduced doses of non-depolarizing neuromuscular blockers and short-acting opioids, tailored to the child's neuromuscular status and respiratory function.

Conflict of interest: None declared.

REFERENCES

1. Sudhakaran R, Unnithan PR, Snehith R. Anaesthetic management of an infant with spinal muscular atrophy [Type 1] for fundoplication and feeding gastrostomy - letter to the Editor. Indian Journal of Clinical Anaesthesia 2023;10(2):214–5. [DOI: 10.18231/j.ijca.2023.044]
2. Halanski MA, Steinfeldt A, Hanna R, Hetzel S, Schroth M, Muldowney. Peri-operative management of children with spinal muscular atrophy. Indian J Anaesth. 2020;64(11):931–6. [DOI: 10.4103/ija.IJA_312_20] [PMID: 33487676]
3. Thomas DE, Sebastian G, Irimpan J, Kumar L. Anesthetic management of a child with spinal muscular atrophy. Amrita Journal of Medicine. 2023;19:147–9. [DOI: 10.4103/AMJM.AMJM_44_23]
4. Akcaalan Y, Erkilic E, Akin M. Anesthesia management in patient with spinal muscular atrophy (SMA) type 2. American Journal of Surgery and Clinical Case Reports. 2022;4(16):1–3.
5. Jang EH, Cho KR, Kim HT, Lim HS, Lee JH, Lee KM, et al. General anesthesia for a spinal muscular atrophy type I patient undergoing feeding gastrostomy. Anesth Pain Med. 2010;5:329–32.
6. Panda S, Rojalin Baby SK, Singh G. Spinal muscular atrophy type II: anesthetic challenges and perioperative management. J Card Crit Care. 2021;5:249–51. [DOI: 10.1055/s-0042-1742401]
7. Brollier LD, Matuszczak M, Marri T, Carbajal JG, Moorman AT, Sorial EM, et al. Anesthetic management of pediatric patients undergoing intrathecal nusinersen administration for treatment of spinal muscular atrophy: a single-center experience. Paediatr Anaesth. 2021;31(2):160–6. [DOI: 10.1111/pan.13964] [PMID: 32623818]
8. Graham RJ, Athiraman U, Laubach AE, Sethna NF. Anesthesia and perioperative medical management of children with spinal muscular atrophy. Paediatr Anaesth. 2009:19(11):1054–63. [DOI: 10.1111/j.1460-9592.2009.03055.x] [PMID: 19558636]
9. Kumar A, Ravi M, Kiran N. Anaesthetic management of patient with spinal muscular atrophy posted for feeding gastrostomy under general anaesthesia. Ind J Anesth Analg. 2024;11(3):143–6. [DOI: 10.21088/ijaa.2349.8471.11324.5]

Анестезија код одојчета са спиналном мишићном атрофијом типа 1 током пласирања вентрикулоперитонеалног шанта

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САЖЕТАК

Увод Спинална мишићна атрофија је наследна болест која се ретко дијагностикује код деце. Најважнији фактори ризика који су повезани са анестезијом код ове деце су повећана осетљивост на опиоидне аналгетике и неуромишићне релаксанте, као и проблеми са обезбеђењем дисајног пута и дисањем. Приказујемо случај осмомесечног женског одојчета код ког је пласиран вентрикулоперитонеални шант.

Приказ болесника Одојчету је планирано пласирање вентрикулоперитонеалног шанта због хидроцефалуса насталог као последица интратекалне инјекције нусинерсена. Уобичајена премедикација бензодиазепинима није дата детету. Изузев спиналне мишићне атрофије, дете није имало других коморбидитета. Општа ендотрахеална анестезија одржавана је инхалационим севофлураном. Детету су дати опиоидни аналгетици, као и рокуронијум у једној дози. По завршетку операције, дете је пробуђено на операционом столу.

Закључак Општа ендотрахеална анестезија код одојчади са спиналном мишићном атрофијом може се безбедно извести. И интравенска и инхалациона анестезија са севофлураном прихватљиве су могућности.

Кључне речи: спинална мишићна атрофија; анестезија; инхалациона анестезија; вентрикулоперитонеални шант

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Suicide in a shooting range – a review of two autopsy cases

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SUMMARY
Introduction Shooting ranges are places designated for firearm shooting practice. Regulations outline the technical and structural requirements, that should ensure the safety of individuals inside the shooting range. Rules also dictate the keeping of user records and firearm-issuance records. However, mandatory medical examinations for shooting-range users are not required, but they are obligatory for obtaining permits for firearm possession and storage.
Outlines of cases We describe two cases of suicide in a shooting range. The individuals were men, whose autopsies were performed at the Institute of Pathology and Forensic Medicine of the Military Medical Academy. In both cases, the cause of death was the destruction of vital brain centers along the trajectory of the gunshot wound.
Conclusion These cases raise the question of whether shooting ranges are truly safe places for training and practice. While technical and structural requirements may be fully met, the health status of individuals who come to these facilities for shooting practice is inadequately monitored.
Keywords: shooting range; firearm; suicide; permit; medical examination

INTRODUCTION

Shooting ranges are intended for the safe handling of firearms, firing a weapon along a single trajectory from a fixed position [1]. A safe person is one who is competent in handling firearms and ammunition, demonstrating that ability at all times [2]. However, suicides do occur in shooting ranges, though reports on such cases are rare. These incidents are revealed during forensic autopsies, making it crucial for forensic pathologists to highlight cases of suicide in shooting ranges and identify possible oversights leading to these incidents. Firearm-training regulations in Serbia are prescribed by the Law on Weapons and Ammunition of the Republic of Serbia (LWARS) and the Rulebook on Firearm Handling Training [3, 4].

So why do suicides still happen in shooting ranges? The execution of suicide requires a motivation to attempt suicide and suicidal disposition. Suicidal stimulants, most commonly alcohol, facilitate the act. Easy access to firearms, combined with these factors, further facilitates the method of suicide. The core issue lies in inadequate monitoring of the health and psychological state of shooting-range users.

The objective of this review is to highlight that shooting ranges are not completely safe places. Suicides do occur in shooting ranges. It is necessary to assess the health condition, primarily the mental state, of individuals who have access to firearms in shooting ranges in order to prevent undesirable events.

REPORTS OF CASES

We describe two cases of suicide that occurred in two different shooting ranges in Belgrade, Serbia. The suicides occurred in the presence of multiple people who were at the shooting range at the time. Both individuals were male and right-handed. In one case, the potential motivation was an unfinished university degree, while the motivation for the second suicide remained unclear.

In the first case, the individual was a 26-year-old man who was not a member of the shooting range. He used the shooting range's services based on a friendship with its employees. He committed suicide one hour and 15 minutes after arriving at the shooting range, using a "CZ 999" pistol, 9 mm caliber, owned by the facility.

In the second case, the individual was a 40-year-old man who was a member of the shooting range and had been attending it regularly for several months. On the day of his suicide, he visited the range in the morning, then returned in the afternoon, committing suicide 15 minutes after his arrival, using a "Magnum Taurus 357" pistol, .357 Mag caliber, owned by the facility. In both cases, the gunshot entrance wound was located in the right temple area, while the exit wound was in the left temple area. The cause of death was the destruction of vital brain centers along the trajectory of the gunshot wound, inflicted by a projectile fired at close range. Toxicological analysis of blood and urine samples taken during autopsy, conducted at the Department of Toxicological Chemistry at

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the Military Medical Academy, did not detect the presence of alcohol. Immunochromatographic (lateral-flow assay) test strips did not reveal the presence of morphine-based opiates, cannabinoids, cocaine, amphetamines, or methamphetamines in the urine samples of either victim. Liquid chromatography with UV spectral detection identified caffeine in the blood and urine samples of the first victim, while paracetamol (0.002 mg/L) was found in the urine sample. No drugs were detected in the samples of the second victim. Gunshot-residue tests on the hands of both examined individuals were positive.

Ethics: The study was approved by the Ethics Committee of the Military Medical Academy (No. 57/2024).

DISCUSSION

The LWARS stipulates that firearm training can be organized by individuals who can provide proof of ownership of shooting ranges, or a lease of this business, possess evidence of the required expertise, and meet the necessary spatial and technical conditions, for safe firearm storage and handling [3]. Technical and construction requirements, such as the width of passageways, rubber strips in front of bullet traps, bulletproof barriers between shooting lanes, evacuation routes, and various other spatial conditions, are outlined in regulations of Serbia and other countries [1, 2, 4]. The risk of poisoning by lead, antimony, and carbon monoxide in shooting ranges is also mitigated through strict control, ventilation, and cleaning protocols [1, 2, 4]. The LWARS states that only adults can obtain a permit to purchase and own firearms. However, the law does not set an age limit for entry into shooting ranges. Some shooting ranges indicate in their presentations that independent use is only permitted for individuals over the age of 18 years [5, 6].

In a retrospective study of forensic autopsies conducted at the Institute of Pathology and Forensic Medicine of the Military Medical Academy from 2010 to 2019, suicides in shooting ranges accounted for 2.25% of all firearm-related suicides. Barber et al. [7] report that shooting range suicides constituted 0.18% of all firearm suicides from 2004 to 2015. Other authors note that most firearm suicides occur in facilities that house both firearm stores and shooting ranges [8]. Due to suicides in shooting ranges in the United States, suicide-prevention measures have been introduced. These measures require that unknown individuals can only visit a shooting range if accompanied by another person, and must provide proof of completed firearm training, or have someone who can confirm their mental competence [7]. Access to firearms increases the risk of suicide in families with a history of suicide cases [9]. It is estimated that stricter government policies on firearm acquisition could reduce firearm-related deaths in the United States by approximately 11% in a single year [10, 11, 12].

Firearm access in shooting ranges is granted to those who hold permits for firearm possession and carrying, as well as to those who do not have such permits. The LWARS establishes the conditions for obtaining firearm possession and carrying permits [3]. Such permits are issued after an assessment of health eligibility, which is determined according to the Rulebook on Health Eligibility Assessment for Civilians for Firearm Possession and Carrying [13]. A clinical examination, hearing and vision tests, and a psychiatric evaluation are required [13]. This rulebook states that alcoholism and psychoactive substance addiction are contraindications for firearm possession. However, it does not mandate a compulsory test for the presence of psychoactive substances. Instead, testing is conducted based on the indication of an occupational medicine physician or neuropsychiatrist, and requires the patient's written consent [13]. The control of alcohol and psychoactive substance use should be mandatory for all individuals with access to firearms. Lower firearm homicide rates have been recorded in countries where firearm access was restricted following penalties for driving under the influence of alcohol or illegal substances [14]. Legal guidelines for issuing firearm permits in the United Kingdom specify that a firearm license will only be granted after obtaining specific information from a physician, regarding the presence of mental-health disorders, neurological diseases, or substance abuse [15].

Suicidal thoughts and behaviors, physical pain, cognitive impairment, depression, anxiety, and psychotic disorders are among the many factors that may lead to suicide [16–19]. The Rulebook on Health Eligibility Assessment in Serbia lists panic states, phobias, sleep disorders, and mood disorders, among various psychiatric and neurological conditions, that are contraindications for firearm possession [13]. It is evident that these disorders must be diagnosed by a physician following a thorough examination. Serbian law stipulates that the authority issuing firearm possession and carrying permits, must inform the patient's primary care physician about the issuance of the permit [3]. The primary care physician is obligated to notify the competent police department about changes in medical conditions and deteriorations in mental health, discovered during examinations [3, 15]. The LWARS also prescribes financial penalties for physicians who fail to comply with this regulation [3]. An individual who holds a firearm possession permit must submit a new medical certificate upon the expiration of the previous one, meaning every five years [3]. This ensures proper monitoring of firearm owners over a specific period. A shooting-range user without a firearm possession permit has not undergone a medical examination, and does not have proof of health eligibility. This means that shooting ranges allow access to firearms for individuals without medical supervision, increasing the likelihood of both suicides and homicides.

Firearm accessibility always carries the risk of unwanted incidents and requires strict control. Although shooting range suicides are rare, proper selection of individuals with firearm access is necessary. Health-eligibility assessments, with a particular focus on mental health and the use of psychoactive substances, should be mandatory for all shooting-range users. This would reduce the risk of suicides and potential homicides in shooting ranges.

Conflict of interest: None declared.

REFERENCES

1. Royal Canadian Mounted Police. Range design and construction guideline [Internet]. Canadian Firearms Program; 2021. Available from: https://publications.gc.ca/site/eng/9.950277/publication.html
2. National Rifle Association. Range design and range safety handbook [Internet]. Bisley (UK): NRA; 2022. Available from: https://nra.org.uk/wp-content/uploads/NRA-RANGE-DESIGN-AND-SAFETY-HANDBOOK-DEC-2022-22-12-22-1.pdf
3. Zakon o oružju i municiji. Službeni glasnik RS. 2015; (20/2015, 10/2019, 14/2022).
4. Pravilnik o obuci i rukovanju vatrenim oružjem, uslovima za bavljenje popravkom i prepravkom oružja i prometom oružja i municije. Službeni glasnik RS. 2016; (13/2016, 88/2016). Available from: http://demo.paragraf.rs/demo/combined/Old/t/t2016_10/t10_0445.htm
5. SKTarget. O nama [Internet]. Available from: https://sktarget.rs
6. Barutana. O nama [Internet]. Available from: https://barutana.rs
7. Barber C, Walters H, Brown T, Hemenway D. Suicides at shooting ranges. Crisis. 2021;42(1):13–9. [DOI: 10.1027/0227-5910/a000676] [PMID: 32343169]
8. Henson-Garcia M, Malthaner L, Beauchamp A, McKay S, Jetelina K. Epidemiological analysis of fatal and non-fatal firearm injuries occurring in gun establishments in the United States, 2015–22. Inj Prev. 2025;31(3):253–6. [DOI: 10.1136/ip-2023-045127] [PMID: 38862213]
9. Favril L, Yu R, Geddes JR, Fazel S. Individual-level risk factors for suicide mortality in the general population: an umbrella review. Lancet Public Health. 2023;8(11):e868–77. [DOI: 10.1016/S2468-2667(23)00207-4] [PMID: 37898519]
10. Kawano B, Agarwal S, Krishnamoorthy V, Raghunathan K, Fernandez-Moure JS, Haines KL. Restrictive firearm laws and firearm-related suicide. J Am Coll Surg. 2023;236(1):37–44. [DOI: 10.1097/XCS.0000000000000431] [PMID: 36519906]
11. Zeoli AM, McCourt AD, Paruk JK. Effectiveness of firearm restriction, background checks, and licensing laws in reducing gun violence. Ann Am Acad Pol Soc Sci. 2023;704(1):118–36. [DOI: 10.1177/00027162231165149]
12. Sharkey P, Kang M. The era of progress on gun mortality: state gun regulations and gun deaths from 1991 to 2016. Epidemiology. 2023;34(6):786–92. [DOI: 10.1097/EDE.0000000000001662] [PMID: 37732847]
13. Pravilnik o utvrđivanju zdravstvene sposobnosti fizičkih lica za držanje i nošenje oružja. Službeni glasnik RS. 2016; (25/2016, 79/2016). Available from: http://demo.paragraf.rs/demo/combined/Old/t/t2016_09/t09_0203.htm
14. Tessler RA, Haviland MJ, Bowen A, Bowen D, Rivara FR, Rowhani-Rahbar A. Association of state-level intoxicated driving laws with firearm homicide and suicide. Inj Prev. 2022;28(1):32–7. [DOI: 10.1136/injuryprev-2020-044052] [PMID: 33687929]
15. Home Office. Firearms licensing: statutory guidance for chief officers of police [Internet]. London: Home Office; 2023. Available from: https://assets.publishing.service.gov.uk/media/68909a84486754ec288783c0/Firearms_licensing_statutory_guidance.pdf
16. Ribeiro JD, Franklin JC, Fox KR, Bentley KH, Kleiman EM, Chang BP, et al. Self-injurious thoughts and behaviours as risk factors for future suicide ideation, attempts, and death: a meta-analysis of longitudinal studies. Psychol Med. 2016;46(2):225–36. [DOI: 10.1017/S0033291715001804] [PMID: 26370729]
17. Smith L, Shin J, Lee S, Oh JW, López-Sánchez G, Kostev K, et al. The association of physical multimorbidity with suicidal ideation and suicide attempts in England: a mediation analysis of influential factors. Int J Soc Psychiatry. 2023;69(3):523–31. [DOI: 10.1177/00207640221137993] [PMID: 36511141]
18. DeVylder JE, Lukens EP, Link BG, Lieberman JA. Suicidal ideation and suicide attempts among adults with psychotic experiences: data from the Collaborative Psychiatric Epidemiology Surveys. JAMA Psychiatry. 2015;72(3):219–25. [DOI: 10.1001/jamapsychiatry.2014.2663] [PMID: 25715312]
19. Favril L, Yu R, Uyar A, Sharpe M, Fazel S. Risk factors for suicide in adults: systematic review and meta-analysis of psychological autopsy studies. Evid Based Ment Health. 2022;25(4):148–55. [DOI: 10.1136/ebmental-2022-300549] [PMID: 36162975]

Самоубиство у стрељани – приказ два случаја аутопсије

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САЖЕТАК

Увод Стрељане су места намењена за гађање ватреним оружјем. Правилником су прописани техничко-грађевински услови за стрељане који би требало да гарантују безбедност лица унутар стрељане. Прописана су и правила о вођењу евиденције корисника стрељане и издавању оружја. Међутим, нису установљени обавезни лекарски прегледи за кориснике стрељана, иако су они обавезни за издавање дозвола за држање и чување оружја.

Прикази случајева Описујемо два случаја самоубиства у стрељани. У питању су мушкарци чије су обдукције обављене на Институту за патологију и судску медицину Војномедицинске академије и у оба случаја узрок смрти било је разарење за живот важних можданих центара дуж канала прострелне главе.

Закључак Поставља се питање да ли су и колико стрељане безбедна места за обуку и вежбе гађања. Техничко-грађевински услови су можда потпуно испуњени, али се здравствено стање особа које долазе у стрељану да гађају неадекватно контролише.

Кључне речи: стрељана; ватрено оружје; самоубиство; дозвола; лекарски преглед

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

Turkish military hospital at the Skull Tower in Niš (1872–1878)

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SUMMARY
With a history that spans 146 years, the military hospital at the Skull Tower in Niš is one of the most esteemed medical institutions of the Army of the Republic of Serbia. However, in previous research about it, it was not sufficiently emphasized that the hospital was, in the same place and with the same function, an institution inherited from the Ottoman era, which ended in Niš in 1878. In Serbian historiography, there are practically no works on this topic. Therefore, we conducted research on the history of this military hospital in the period under the Ottomans. In that way, we achieved the goals of our research: to establish a direct connection between the Ottoman and Serbian military hospitals and to move the founding date of the military health institution at the Skull Tower further into the past. In addition, the research also led us to findings that challenge the established opinion in Serbian historiography that in Niš, during the last decades under the Ottomans, there were no highly educated health personnel and that modern medicine was not practiced there during that time. To achieve all this, we used unpublished Ottoman archival materials, narrative sources and Ottoman press from the 19th century, as well as the works of various scientific formats by contemporary Serbian, Turkish, and European authors.
Keywords: Niš; Ottomans; healthcare system; army; hospital; 19th century

INTRODUCTION

After the Principality of Serbia took over Niš in 1878, a new era began in the history of this city. Aside from the political and urban changes, the city also went through a cultural metamorphosis in which the Ottoman Westernization of Niš was replaced by Europeanization under the Obrenović dynasty [1]. However, several institutions from the Ottoman era not only survived those changes, but also evolved in such a way that they almost retained their original function and continued to exist under the new authorities. One such institution is the military hospital at the Skull Tower.

Ottoman military medicine is a topic that is generally not sufficiently explored even by Turkish researchers, so the information about the military hospital at the Skull Tower is extremely scarce. That is why we had to rely on indirect data in order to reconstruct its work. In addition, we consulted sources on public health in the Ottoman Empire and analyzed the state of healthcare in Ottoman Niš during the 19th century to project the functioning of the hospital and to evaluate its importance.

THE ISSUES OF PUBLIC HEALTH IN OTTOMAN NIŠ DURING THE 19th CENTURY

During the 19th century under the Ottomans, Niš was a particularly colorful community of various ethnicities and religions. As opposed to politics and religion, the domain in which they all voluntarily embraced each other's influence was folk medicine or, to be more precise, a mixture of phytotherapy, folklore, and superstition.

The Muslims in Niš were inclined to mysticism and folklore of other religious groups due to the great popularity of the Bektashis in this city. The theosophy of this dervish order was a mixture of mysticism, folklore, and Islamic and Judeo-Christian beliefs [2], which helped develop the cults of several local Muslim saints to whom Muslims used to pray for aid [3].

The local Christian population was traditionally treated with various herbs that were picked on major religious holidays or consecrated in churches during the ceremonies. When the herbs did not provide a solution, the people turned to superstition and quacks, who treated more with autosuggestion than with proper medical intervention. Ottoman Niš in the 19th century was full of such "experts": Muslim priests who made magical writings and amulets, Christian monks who read special prayers, self-taught midwives and bone-setters, barbers and tin artisans, spell-casters, and self-proclaimed pharmacists who treated all possible diseases with their strange remedies [4, 5]. All of them offered solutions to combat the omnipresent invisible demons and spirits (bacteria and germs) that brought disease and death. The remedies and treatment methods of these people were equally obscure, ranging from the use of herbs that actually have

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medicinal value to animal feces, human urine and dust from craftsmen's shops [4, 5].

Naturally, such an ignorant understanding of health was reflected in public hygiene. The streets were narrow and the districts dark and airless, always shaded by overgrown gardens and lush trees full of mosquitoes. The artisans used to dump wastewater in front of their shops [6]. Inns and taverns often did not have stables, so the feces of draft cattle and horses remained around these buildings, causing a bad smell and dense swarms of flies. Septic tanks in households were dug shallowly and, when overflowed, they were simply buried and new ones were dug nearby. There were 17 public fountains in the city. Most of them had poorly maintained pipelines, so during heavy rains the water from them was full of mud, rot and insects [7]. Private wells were not in better condition, either. Households usually burned garbage on Saturday evenings, so the whole city was enveloped in thick smelly fog for a day [7]. Domestic animals moved freely, so it was normal for their half-decomposed corpses to be found everywhere [6, 8].

All this caused Niš to be an epidemic hell, a community where deaths from tuberculosis, venereal and intestinal diseases, malaria, dysentery, scarlet fever, and typhoid were an accepted everyday occurrence for decades [5, 7, 9].

THE OTTOMAN PUBLIC HEALTHCARE SYSTEM IN THE 19th CENTURY

The development of modern public healthcare mechanisms in the Ottoman Empire began with Sultan Selim III (1789–1807) and his military reforms [10]. With the aim of modernizing the Ottoman army according to the European model, the sultan began to develop the services that should take care of it, including its health. In this regard, the first Ottoman military hospital was founded in 1799 under the name Levent Estate Hospital (*Levent Çiftliği Hastahanesi*) [11]. It worked in parallel with classical public hospitals (*dâr-üş şifâ*) and, like the rest of Selim III reforms' outcomes, was promoted in different regions of the country over time. A system of barracks of the modernized Ottoman army was developed with the hospital department attached to them. This system of military barracks was maintained throughout the 19th century, and in many provincial centers (including Niš), these military hospital departments would be responsible for local public healthcare.

The first modern Ottoman medical school was founded in 1805 under the name Medical School at the Imperial Arsenal [12]. It provided not only medical education, but also medical treatments, working that way as some sort of a clinic. However, the school burned down in 1822 and was left as such for several years.

A further enhancement of the Ottoman medical system was the foundation of the Imperial Medical College in 1827 in Constantinople [13]. In the following decades, this higher-education institution grew, changed its name and location, raised the quality of teaching, and developed courses for numerous branches of medicine. By the middle of the 19th century, it had become a custom for the lecturers to be distinguished experts from the countries of Central and Western Europe, and for the courses to be conducted in French, as complex and modern as in Europe at that time [13, 14]. Although the sultans and the State generously supported the work of the college, throughout the 19th century the staff it trained was not numerous enough to visibly contribute to the health picture of the Ottoman state, especially in the provinces. The Crimean War (1853–1856) further emphasized the need for trained doctors at the state level, but the percentage of applicants to the Imperial Medical College was chronically low. The reasons for that were the social structure of Ottoman society and the European character of the college's program, which was too alien and challenging to most potential candidates. This situation forced the State to tolerate the work of quacks and questionable practitioners in the provinces. However, in order to distinguish valid physicians from mere quacks, the Law on the Practice of Medicine in the Provinces (1861) mandated that all of them must have certificates and licenses to practice medicine or pharmacy [13]. Filtering provincial quacks in this way, the State referred people in the provinces to the services of trained doctors.

MEDICAL STAFF AND CAPACITIES IN NIŠ BEFORE 1872

Although the population relied on quacks and folk healers, the local authorities did a lot to ensure there were always trained doctors in Niš and capacity for proper medical treatment. For this reason, we challenge the findings of some Serbian researchers and claims in official monographs about the military hospital at the Skull Tower, that there were no civilian or other trained doctors in Niš before 1878 [5, 7, 8, 15].

According to the published narratives from the 19th century, trained doctors were present in Niš as early as 1829 [16]. Also, the holdings at the Archives of Presidency of the Republic of Turkey (BOA), such as *Sadaret Mektubi Kalemi Evrakı*, *Mühimme Kalemi Evrakı*, *Hariciye Nezareti Mektubi Kalemi*, *Hariciye Nezareti Tercüme Odasi*, *Sadaret Umum Vilayat Evrakı*, *Meclis-i Vala*, and *Irade Dahiliye* preserve numerous documents which confirm the presence of the official doctors at Niš until 1878. Often, they were not given the same position or qualifications – some were mentioned as surgeons, city doctors (*Niş kasaba tabibi*), military doctors, or simply as doctors [16–20]. For some of them it is mentioned that they were trained at the Imperial Medical College in Constantinople [17, 21], but for the others the information about their education is lacking. Not all the doctors were Muslims. Many of them were native Ottoman Slavs, Jews, or Greeks [4, 17], some even Europeans (Austrians, Italians, French) who sought the service on the Ottoman side [16, 22]. Although their services lasted in average from one to three years, the point is

that Niš has never been without an educated doctor from 1830s until 1878. Therefore, every civilian or soldier who trusted them (or who could afford them) had a chance to receive medical treatment appropriate for that time.

As for the medical institutions, in 1878 the Serbian authorities in Niš inherited the hospital on the Leskovac Road, Islahana, and the large hospital at the Skull Tower [8, 9, 15]. The first was actually the infirmary of the modern military barracks under Bubanj Hill (the so-called New Barracks), which Midhat Pasha built in 1862 [23]. Islahana was an orphanage and a craft school, which was adapted into a hospital on the eve of the Serbian–Ottoman wars (1876–1878). The third building was the only one built for the purpose and with the specialized facilities to provide professional medical help, hence, a hospital in the true sense.

THE BUILDING OF THE MILITARY HOSPITAL AT THE SKULL TOWER

In search for primary material about this hospital for the period 1872–1878, we looked into the Archives of Presidency of the Republic of Turkey (BOA) and examined about 10 000 pages of Ottoman press from the 19th century. We did not manage to find a single archival document directly related to this building before 1878, though it is certain that Serbian authorities in 1883 handed over to the Ottoman counterpart all documentation found in the Ottoman hospitals in Niš [24]. We managed to solve this obstacle by consulting the sources, narratives and literature that provide indirect information. That way we successfully reconstructed the capacities and conditions in which this institution worked. The large military hospital in Niš was built right next to the Skull Tower, the edifice which the governor of Niš Hurshid Pasha built after the victory over the Serbian insurgents on Čegar in 1809. This location was suitable for technical, financial, and logistical reasons.

The main part of the building that was used for the hospital was actually a summer residence owned by the successors of Hafis Pasha, an Ottoman commander and the governor of Niš who died on Ivankovac (1805) fighting the Serbian insurgents [25]. Since the Ottoman government in the late Tanzimat (1853–1876) preferred to buy off existing buildings, instead of building the new ones from scratch, from the point of saving costs it was quite understandable why spacious (and neglected) buildings like a residence on the outskirts of Niš were a financially suitable solution.

Logistically, the hospital in this place was suitable because of the proximity of the section of the Via Militaris which led from Niš to Sofia. In this way, the hospital was located at the very opposite end of Niš from which it could be in danger in the case of a war with the Principality of Serbia. In addition, the spacious field that stretched from the Skull Tower to Niška Banja to the east offered the possibility, in case of need, to erect additional barracks directly around the hospital for the reception of the sick and wounded, without being hindered by the dense city infrastructure. Also, to the west of the Skull Tower, in the direction toward the center of Niš, there was a colony of Circassian and Tatar refugees from the Crimea. The hospital near the Skull Tower could directly meet their health needs. With all this, we should not ignore the fact that the hospital in this part of the outskirts of Niš, with its human nature, pacified the terrible Skull Tower, a building that spread a bad name about the Ottomans in Europe and which Midhat Pasha, the governor of Niš, wanted to remove in the 1860s [25].

What other conditions the Ottoman engineers used to take into consideration when building the hospitals can be seen from the report of certain Major Tosun Effendi. In 1860 he was engaged to inspect the location for building a hospital in Priština. For the plot of land he found suitable for a hospital in his report he wrote that it was on the meadow ("vast, green and suitable for growing garden"), located in the upper part of the town (secure from floods), it was three to five minutes away from the downtown (meaning, from the noise, dirt, and bad smell from overcrowded districts), supplied with running water (streams or creeks) and with mild micro-climate [12]. The location at the Skull Tower was simply perfect by all these standards, with one exception. The creek Gabrovačka Reka, which passes only 100 m from the Skull Tower, had weak water flow, so it was most likely used only for removal of waste and wastewater from the hospital. What is certain is that the Serbian hospital after 1878 used the creek for that purpose [26]. Ottoman engineers solved the supply of drinking water in some other way.

Due to the lack of archive material, especially budget and inspection ledgers *(keşif defters)*, it is impossible to reliably estimate the costs of building the hospital. Based on preserved documents on the construction of military hospitals in the Balkan provinces since the 1860s and the funds Ottoman authorities spent for the construction of complex institutions in Niš in the 1860s and 1870s (Islahana, telegraph station, the New Barracks, Serbian school), the expenses might have stretched from 116,000 up to 860,000 piasters [12, 25]. This is where our further speculation ends.

Finally, on May 1, 1872, a hospital, designed by Major Osman Effendi from Sofia, was opened [27]. The ceremonial opening was attended by the then-governor of Niš, Ali-Riza Pasha, and, as was the protocol, representatives of the high city administration and religious communities in Niš, notable citizens and a numerous crowd.

According to Osman Effendi's project, the hospital had two floors. On the ground floor, there were two sickrooms, a room for orderlies, an operating room, a pharmacy, a storage room for clothes, a pantry room, a kitchen and a bathroom. The second floor had two sickrooms with 88 beds, two rooms for servants, a pharmacy lab, a room for the orderlies, a visiting room and a special room for women who had recently given birth [25].

Since the hospital was the largest medical institution in the County of Niš, and from the very beginning, it had complex units (operating room, pharmacy, laboratory),

performed deliveries and took care of bedridden patients, we can speculate what other ancillary facilities must or could have been part of the hospital complex. For this, we used the organization plan of the Imperial Medical College in Constantinople (from the second half of the 19th century), which was the best equipped medical institution in the Ottoman Empire [12]. According to our estimates, because of the pharmaceutical laboratory where the drugs were prepared, the hospital must have had some kind of greenhouse or botanical garden. Also, the spacious field around it made it possible to have a garden for convalescents to walk and to grow fruits and vegetables for feeding the sick. A barn was also necessary for the latter. If patients were not fed in sickrooms, the hospital probably had some kind of canteen or messroom. As the largest hospital in the county, this institution could also have had its own morgue. The operating room and the surgeons in the hospital's permanent staff allowed for the possibility that the autopsies could be performed as part of the investigative procedure; however, this is questionable due to the unpopularity of this procedure among the Muslim population from the religious perspective. Of the auxiliary facilities, the hospital certainly had a reservoir with accumulated water for the maintenance of sanitary conditions and fire prevention, a firewood and coal shed, a basement, etc.

From all this it is obvious that the hospital right from the start was not intended for the military staff only, but to provide healthcare for the entire civilian population, as well. Regarding that, we even have statistics for the first year of its work (March 1872 – August 1873). According to these statistics, during the first year, the hospital treated 311 male and 293 female patients, of which 21 men and 3 women died [27]. The newspaper article from *Dunav/Tuna gazetesi* did not specify which territory these numbers refer to, whether it is only the city of Niš or the entire county. Also, it is unknown what was the ratio between the patients who were treated in the hospital and those who did it at home, using other methods. The only conclusion is that according to the number of patients who died (only 4 %), the hospital undeniably contributed to the quality of health of the local population.

The circumstances during the Serbian–Ottoman wars completely exhausted the capacities of the hospital, so the Serbian authorities in 1878 found it neglected and ruined. The head of the Serbian Military Medical Service, Dr. Vladan Đorđević, visited the hospital during the very first days after the Serbian army took Niš. He did not leave a description of the state in which he found it, but indirectly stated that it was in the same condition as the hospital at the New Barracks: sheets and blankets were dirty beyond any acceptance, the patients were left neglected and without any professional supervision, the floors and corridors were muddy and contaminated heavily with filth, pharmacy stocks in disarray and the medical utensils scattered or stolen [28]. However, a lot of hospital materials and supplies were still there, which convinced Dr. Đorđević that it was indeed a healthcare facility, so it could be still used as such.

The importance of the hospital at the Skull Tower was already known to Serbian authorities. Therefore, the Serbian government invested funds in its renovation and immediately returned it to its original function. The process was initiated by Dr. Đorđević himself. On January 7, 1878, he submitted a proposal to the Serbian Supreme Military Staff to form a system of a Great Military Hospital in Niš, consisting of the hospital at the Skull Tower, Islahana, and the New Barracks. The Serbian Ministry of Finance approved the funds for the initiative on January 9, and the very next day the new medical system in Niš began operating [28]. The Great Military Hospital in Niš was organized in six medical wards. The First ward was located at the hospital at the Skull Tower, which indicates the importance this facility had for the new authorities. A large complex of supporting facilities was built around it, and the hospital was handed over to the Chief Physician Dr. Đorđe Dimitrijević, to run it [29]. This completed the transfer of the hospital from the Ottoman to Serbian management and started a new era in its work, to become a reputable institution of Serbian military healthcare system, which lasts to this day.

CONCLUSION

Due to historical circumstances, before 1878 Niš was exempted from the modernization that the Serbian state was going through at that time, but in the same period it was included in the strategy of adopting European novelties implemented by the Ottoman state. The most visible evidence of this is in the matters of general public interest, such as public health. In this regard, the results of our research proved that the Ottoman hospital at the Skull Tower was the first primary healthcare institution in Niš and the predecessor of the Serbian military hospital founded at the same place in 1878. The historical facts are that these institutions had different organization and technical conditions; moreover, they belonged to different states and governing systems. But they also had the same primary function and the importance for the local community, which makes them the sole institution that actually belongs to two different eras.

The milestone of evolution that the hospital at the Skull Tower has gone through was the year 1878. Although with the capacities crippled by the Serbian-Ottoman wars, the hospital at that time still had potential to evolve further. Its position, capacities and strategic value, which the Ottomans set, made it easy for Serbian Dr. Vladan Đorđević to act swiftly and turn the hospital at the Skull Tower into what it is today. Due to historical circumstances, we will never learn whether the Ottomans also had such ambitious plans for the hospital after 1878. The only certain things are the straight historical continuity in the functioning of the hospital under Ottoman and Serbian authorities and the fact this institution moves the foundation of modern healthcare in Niš deeper into the past. That way, in the wider scope, the hospital at the Skull Tower

contributes to heterogeneity (therefore, the cosmopolitan character) of the historical heritage of this city.

Ethics: The authors declare that the article was written according to the ethical standards of the Serbian Archives of Medicine as well as ethical standards of institutions for each author involved.

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REFERENCES

1. Ranđelović M. Tragovi osmanske kulture u urbanoj infrastrukturi i živoj zajednici obrenovićevskog Niša (1878–1903). Glasnik Etnografskog instituta SANU. 2024;72(1):133–54. [DOI: 10.2298/GEI2401133R]
2. Ranđelović M. Bektašije. Religija i Tolerancija. 2023;21(40):267–85. [DOI: 10.18485/rit.2023.21.40.5]
3. Ranđelović M. Zahide Badži. Jedan primer sufijske mistike iz osmanskog Niša. Glasnik Etnografskog instituta SANU. 2018;66(1):139–54. [DOI: 10.2298/GEI1801139R]
4. Nikolić V. Iz narodne medicine u Nišu za vreme turske vladavine. Glasnik Etnografskog muzeja u Beogradu. 1940;15:41–8.
5. Stamenković Đ. 100 godina farmacije u Nišu 1878–1978. Niš: Zdravstvena radna organizacija udružene apoteke Niš; 1978.
6. Ranđelović M. Zapisi o Nišu (1877–1914): odlomci iz pisama, dnevnika, putopisa i memoara. Beograd: Magelan Press; 2014.
7. Milojević V. Zdravstvena služba i zaštita od 1878. do 1941. In: Milić D, editor. Istorija Niša. Vol 2. Niš: Gradina–Prosveta; 1984. p. 497–526.
8. Paunović S. 110-godišnjica Vojne bolnice u Nišu. Niš: Vojna bolnica; 1988.
9. Živić RV. Knjiga o bolnici. Niš: Prosveta; 2002.
10. Yılmaz H. İhtilal Diplomasisi ve Osmanlılar, 1809 Çanakkale Antlaşması. İstanbul: Kitapevi; 2021.
11. Stanford S. Eski ve Yeni Arasında Sultan III. Selim Yönetiminde Osmanlı İmparatorluğu. İstanbul: Kapı; 2008.
12. Babuçoğlu M, Özdil O, Karakuş SE. Osmanlı belgelerinde askeri tip ve Balkan askeri hastaneleri. Ankara: Gülhaneliler Eğitim Sağlık ve Sosyal Dayanışma Vakıfı; 2013.
13. Rasimoğlu-İlikan CG. Boundaries, education and licence: the nineteenth-century Ottoman standardization of medical professions. Trakya Üniversitesi Sosyal Bilimler Dergisi. 2017;19(1):227–45.
14. Ulman YI. Medical modernization in 19th-century Ottoman Empire with special reference to the introduction of Roentgen rays in Turkey. In: Moulin AM, Ulman YI, editors. Perilous Modernity: History of medicine in the Ottoman Empire and the Middle East from the 19th century onwards. Istanbul: The ISIS Press; 2010. p. 105–18. [DOI: 10.31826/9781463230005-009]
15. Milenković S, Dimić M. 125 godina Vojne bolnice u Nišu. Niš–Zrenjanin–Bečej: Vojna bolnica; Jugomedija; Proleter; 2003.
16. Ranđelović M. Zapisi o Nišu (269–1877). Niš: Medivest; 2013.
17. Governor of Niš to Grand Vezier. 1849 Jul 1. T.C. Cumhurbaşkanlığı Devlet Arşivleri Başkanlığı (BOA), Mühimme Kalemi Evrakı fonds, A.MKT.MHM-15-23-1-1.
18. Order from Grand Vezier to Chief Doctor of the Ottoman Empire. 1849 Oct 4. BOA, Sadaret Mektubi Kalemi Evrakı fonds, A.MKT-228-10-1-1.
19. Letter from Grand Vezier to the Principal of Imperial Medical College. 1861 May 25. BOA, Mühimme Kalemi Evrakı fonds, A.MKT. MHM-220-41-1-1.
20. Sultan Abdulaziz's approval for sending Major Dr Omer Effendi to the military hospital at Niš. 1875 Sep 17. BOA, Irade Dahiliye fonds, I.DH-707-49524-3-1.
21. Letter from Grand Vezier to the Governor of Niš. 1861 Oct 27. BOA, Sadaret Umum Vilayat Evrakı fonds, A.MKT.UM-510-63-1-1.
22. Letter from the Austrian diplomatic representative to the Sublime Porte. 1853 May 10. BOA, Hariciye Nezareti Tercüme Odası, HR.TO-153-31-1-1.
23. Ranđelović M. The toponyms of Ottoman Niš: the New barracks under Bubanj Hill. Zbornik radova Filozofskog fakulteta Univerziteta u Prištini. 2024;54(3):32–43. [DOI: 10.5937/zrffp54-48726]
24. Letter from the Ministry of the Interior to Imperial General Staff Headquarters. 1883 Jul 20. BOA, Hariciye Nezareti Siyasi fonds, HR.SYS-1489-59-3-1.
25. Ranđelović M. Osmanski upravnici Niša u XIX veku 1799–1878. Niš: Scero Print; 2022.
26. Stanojević V. Istorija srpskog vojnog saniteta. Naše ratno sanitetsko iskustvo. Beograd: Vojnoizdavački i novinski centar; 1992.
27. Dopiska ot Niš. Dunav/Tuna. 1874 Oct 16; Sect A2 (col. 2).
28. Đorđević V. Istorija srpskog vojnog saniteta III. Beograd: Državna štamparija; 1880.
29. Popović Filipović S. Čuvari narodnog zdravlja u Nišu u ratu i miru (1878–1941). Beograd: Medija centar „Odbrana"; 2022.

Турска војна болница код Ћеле-куле у Нишу (1872–1878)

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САЖЕТАК

Са историјом која траје већ 146 година, војна болница код Ћеле-куле у Нишу једна је од најугледнијих здравствених установа Војске Републике Србије. Међутим, у досадашњим истраживањима о њој недовољно се наглашавало да је болница била, на истом месту и са истом функцијом, наслеђена институција из османске епохе, која је у Нишу окончана 1878. године. У српској историографији радова на ту тему практично нема. Управо због тога спровели смо истраживање о историји ове војне болнице у периоду под Османлијама. На тај начин остварили смо циљеве нашег истраживања: утврдили смо директну везу између османске и српске војне болнице (као и здравствене праксе која се у обе спроводила) и померили дубље у прошлост годину оснивања војне здравствене установе код Ћеле-куле. Осим наведеног, истраживање нас је довело и до сазнања која оспоравају уврежено мишљење у српској историографији да у Нишу током последњих деценија под Османлијама није било високообразованог здравственог кадра, као и да се у граду за то време није спроводила савремена медицинска пракса.

У циљу постизања свега наведеног, за истраживање је коришћена необјављена османска архивска грађа, наративни извори и османска штампа из 19. века, као и дела различитог научног формата савремених српских, турских и европских аутора.

Кључне речи: Ниш; Османлије; систем здравствене заштите; војска; болница; 19. век

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PERSONAL VIEW ARTICLE / ЛИЧНИ СТАВ

AI in science – dusk or dawn?

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SUMMARY
The peer review process remains a cornerstone of scientific integrity, ensuring that research findings are critically evaluated before entering the scientific record. With the growing integration of artificial intelligence (AI) and the widespread adoption of large language models (LLMs) such as ChatGPT, the research and publishing landscape is undergoing rapid transformation. While AI offers considerable advantages – enhancing efficiency in manuscript drafting, editing, and preliminary evaluation – it also introduces significant risks, particularly when used beyond its optimal scope. This viewpoint underscores the limitations of generative AI, including the phenomenon of "hallucinated" references and the inability to perform genuine critical thinking. These shortcomings raise serious concerns about the validity of scientific content when AI is used without appropriate human oversight. Emphasis is placed on preserving the human-centered nature of peer review, which is vital to safeguarding scientific credibility. In doing so, this article reinforces the necessity of evolving editorial and publishing policies, such as Elsevier's updated guidelines on the use of generative AI, to ensure responsible integration of these technologies into the research ecosystem.
Keywords: artificial intelligence; ChatGPT; large language models; peer review

The peer review process has long served as a cornerstone of scientific integrity, ensuring that manuscripts undergo rigorous evaluation by experts before publication. This system not only validates methodological soundness and scientific merit but also provides reassurance to clinicians and policymakers that published findings can be reliably integrated into evidence-based medical practice. However, despite its value, peer review is not without limitations – chiefly, the time-consuming nature of the process and the inherent risk of cognitive and personal biases [1].

With the advent of artificial intelligence (AI) and, more recently, large language models (LLMs) such as ChatGPT, there has been a growing temptation to streamline the scientific publishing pipeline. These tools offer appealing solutions to common barriers in scientific communication: drafting outlines, overcoming writer's block, performing rapid literature summarization, and even translating or proofreading manuscripts in record time [2, 3]. Yet, while the capabilities of AI are undeniably impressive, this raises a critical question: what are the limitations and implications of integrating generative AI into the publication workflow, particularly in the domain of peer review?

At present, many leading publishers, including Elsevier, Springer Nature, and JAMA Network, have established formal policies governing the use of generative AI in scientific writing and peer review [4–8]. These policies often emphasize transparency, discouraging unacknowledged AI authorship and warning against reliance on AI-generated content without human validation. The core concern underpinning these restrictions is the phenomenon known as "AI hallucination" – the generation of plausible-sounding but factually incorrect information [9, 10, 11].

This phenomenon poses a serious threat to the dissemination of accurate scientific knowledge. In medicine, where publications directly inform clinical guidelines and therapeutic decisions, the presence of fabricated facts or references can be detrimental. For example, ChatGPT may synthesize text that appears authoritative, complete with fabricated citations and erroneous data, despite having no access to real-time medical databases such as PubMed or updated literature past its training cut-off [9–16]. Even in newer, premium LLMs that are equipped with internet access, the generated references are frequently hallucinatory – fabricated altogether or inserted as placeholders with no meaningful connection to the supported claim. In some instances, the cited reference may be real but entirely unrelated to the content it is purported to substantiate, introducing a false sense of credibility and potentially misleading readers who do not perform manual verification.

Consequently, the uncritical use of such models risks introducing misinformation into the scientific corpus, potentially undermining clinical care and public trust [9, 17].

The peer review process is particularly vulnerable to this dynamic. While AI may be leveraged to assist in administrative triage (e.g., verifying submission completeness or adherence to formatting guidelines), its integration into substantive manuscript evaluation

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introduces the risk of dehumanizing a process built upon expert judgment and critical analysis. Peer review is not merely a procedural checkpoint but a cognitive exercise that demands synthesis, skepticism, contextualization, and the application of domain-specific expertise – capabilities that current AI lacks [1, 18].

LLMs such as ChatGPT generate responses based on statistical associations in training data, rather than through genuine comprehension or deductive reasoning. These models operate through token prediction, optimizing linguistic fluency rather than scientific validity [19]. In contrast, human reviewers draw upon a lifetime of experience, ethical reasoning, and real-world understanding of clinical implications – tools that no model, regardless of its complexity, can replicate. Thus, the substitution of human reviewers with AI compromises the foundational purpose of peer review and threatens the gatekeeping function that upholds scientific quality [20].

It is also critical to highlight that the over-standardization introduced by AI-driven manuscript screening or review can discourage novel or paradigm-shifting research. Homogenized feedback, patterned on previous outputs, may suppress the diversity of scientific thought and innovation. Moreover, inappropriate rejection of unconventional but methodologically sound work could prevent important advances from entering the academic discourse.

In light of these concerns, AI should be viewed as an augmentative – not autonomous – tool. It is well-positioned to assist authors in drafting, editing, or organizing manuscripts, and may be used for non-substantive tasks such as checking grammatical accuracy or enhancing language clarity [2, 3]. However, as manuscripts progress through submission and into review, reliance on AI should be consciously minimized to preserve the essential human elements of critique, reflection, and accountability.

In conclusion, while the incorporation of AI in medical research and publishing offers significant promise for increasing efficiency, its use must be bounded by ethical considerations and guided by firm human oversight. Misuse of generative AI risks undermining the reliability of the scientific literature, particularly in medicine, where lives may depend on the accuracy of published findings. Therefore, safeguarding peer review as a human-driven process remains paramount to maintaining the credibility, rigor, and ethical integrity of scientific discourse.

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REFERENCES

1. Tennant JP, Dugan JM, Graziotin D, Jacques DC, Waldner F, Mietchen D, et al. A multi-disciplinary perspective on emergent and future innovations in peer review. F1000Res. 2017;6:1151. [DOI: 10.12688/f1000research.12037.3] [PMID: 29188015]
2. Huang J, Tan M. The role of ChatGPT in scientific communication: writing better scientific review articles. Am J Cancer Res. 2023;13(4):1148–54. [PMID: 37168339]
3. Blanchard F, Assefi M, Gatulle N, Constantin JM. ChatGPT in the world of medical research: from how it works to how to use it. Anaesth Crit Care Pain Med. 2023;42(3):101231. [DOI: 10.1016/j.accpm.2023.101231] [PMID: 37030395]
4. Elsevier. Generative AI Policy for Journals [Internet]. 2025. Available from: https://www.elsevier.com/about/policies-and-standards/generative-ai-policies-for-journals.
5. Elsevier. The use of generative AI and AI-assisted technologies in the review process for Elsevier [Internet]. 2025. Available from: https://www.elsevier.com/about/policies-and-standards/the-use-of-generative-ai-and-ai-assisted-technologies-in-the-review-process.
6. Elsevier. The use of generative AI and AI-assisted technologies in writing for Elsevier [Internet]. 2025. Available from: https://www.elsevier.com/about/policies-and-standards/the-use-of-generative-ai-and-ai-assisted-technologies-in-writing-for-elsevier.
7. Springer Nature. Editorial Policies [Internet]. 2025. Available from: https://www.springernature.com/gp/policies/editorial-policies.
8. Flanagin A, Bibbins-Domingo K, Berkwits M, Christiansen SL. Nonhuman "authors" and implications for the integrity of scientific publication and medical knowledge. JAMA. 2023;329(8):637–9. [DOI: 10.1001/jama.2023.1344] [PMID: 36719674]
9. Alkaissi H, McFarlane SI. Artificial hallucinations in ChatGPT: implications in scientific writing. Cureus. 2023;15(2):e35179. [DOI: 10.7759/cureus.35179] [PMID: 36811129]
10. Homolak J. Opportunities and risks of ChatGPT in medicine, science, and academic publishing: a modern Promethean dilemma. Croat Med J. 2023;64(1):1–3. [DOI: 10.3325/cmj.2023.64.1] [PMID: 36864812]
11. Jones N. AI hallucinations can't be stopped – but these techniques can limit their damage. Nature. 2025;637(8047):778–80. [DOI: 10.1038/d41586-025-00068-5] [PMID: 39838050]
12. Biswas S. ChatGPT and the future of medical writing. Radiology. 2023;307(2):e223312. [DOI: 10.1148/radiol.223312] [PMID: 36728748]
13. Temsah O, Khan SA, Chaiah Y, Senjab A, Alhasan K, Jamal A, et al. Overview of early ChatGPT's presence in medical literature: insights from a hybrid literature review by ChatGPT and human experts. Cureus. 2023;15(4):e37281. [DOI: 10.7759/cureus.37281] [PMID: 37038381]
14. Cascella M, Montomoli J, Bellini V, Bignami E. Evaluating the feasibility of ChatGPT in healthcare: an analysis of multiple clinical and research scenarios. J Med Syst. 2023;47(1):33. [DOI: 10.1007/s10916-023-01925-4] [PMID: 36869927]
15. Asgari E, Montaña-Brown N, Dubois M, Khalil S, Balloch J, Yeung JA, et al. A framework to assess clinical safety and hallucination rates of LLMs for medical text summarisation. NPJ Digit Med. 2025;8(1):274. [DOI: 10.1038/s41746-025-01670-7] [PMID: 40360677]
16. Roustan D, Bastardot F. The clinicians' guide to large language models: a general perspective with a focus on hallucinations. Interact J Med Res. 2025;14:e59823. [DOI: 10.2196/59823] [PMID: 39874574]
17. Rozencwajg S, Kantor E. Elevating scientific writing with ChatGPT: a guide for reviewers, editors and authors. Anaesth Crit Care Pain Med. 2023;42(3):101209. [DOI: 10.1016/j.accpm.2023.101209] [PMID: 36871626]
18. Chalmers I, Glasziou P. Avoidable waste in the production and reporting of research evidence. Lancet. 2009;374(9683):86–9. [DOI: 10.1016/S0140-6736(09)60329-9] [PMID: 19525005]
19. OpenAI. ChatGPT: Optimizing language models for dialogue [Internet]. 2022. Available from: https://openai.com/blog/chatgpt/.
20. Thorp HH. ChatGPT is fun, but not an author. Science. 2023;379(6630):313. [DOI: 10.1126/science.adg7879] [PMID: 36701446]

Вештачка интелигенција у науци – сумрак или зора?

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САЖЕТАК

Процес рецензије представља темељ научне валидности, осигуравајући да резултати истраживања буду критички процењени пре објављивања. Са све већом интеграцијом вештачке интелигенције и широком доступношћу великих језичких модела (*Large Language Models – LLMs*), попут *ChatGPT*-а, научноистраживачки и издавачки процес пролазе кроз значајне промене. Иако вештачка интелигенција доноси бројне предности – побољшање ефикасности у писању, уређивању и почетној евалуацији рукописа – њена примена изван тих оквира носи озбиљне ризике. Овај рад указује на ограничења генеративне вештачке интелигенције, укључујући појаву „халуцинираних“ референци и недостатак способности за критичко размишљање, што може угрозити научну поузданост када се ови алати користе без одговарајућег људског надзора. Посебан акценат стављен је на очување људске улоге у процесу рецензије као кључне карике у очувању кредибилитета науке. У том контексту, аутори подржавају потребу за ажурирањем издавачких политика, попут нових смерница издавачке куће *Elsevier* о употреби генеративне вештачке интелигенције, како би се осигурала одговорна и етичка интеграција ових технологија у научноистраживачки екосистем.

Кључне речи: вештачка интелигенција; *ChatGPT*; велики језички модели; рецензија

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 хонорарашу. 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. Примере 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 износи 6.000 динара по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада домаћих аутора износи 12.000 динара по раду;
- накнада за преглед сваког примљеног рада страних аутора износи 75 евра, или динарска противвредност по средњем курсу НБС на дан плаћања, по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада страних аутора износи 150 евра, или динарска противвредност по средњем курсу НБС на дан плаћања, 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:
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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).

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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.

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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.

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