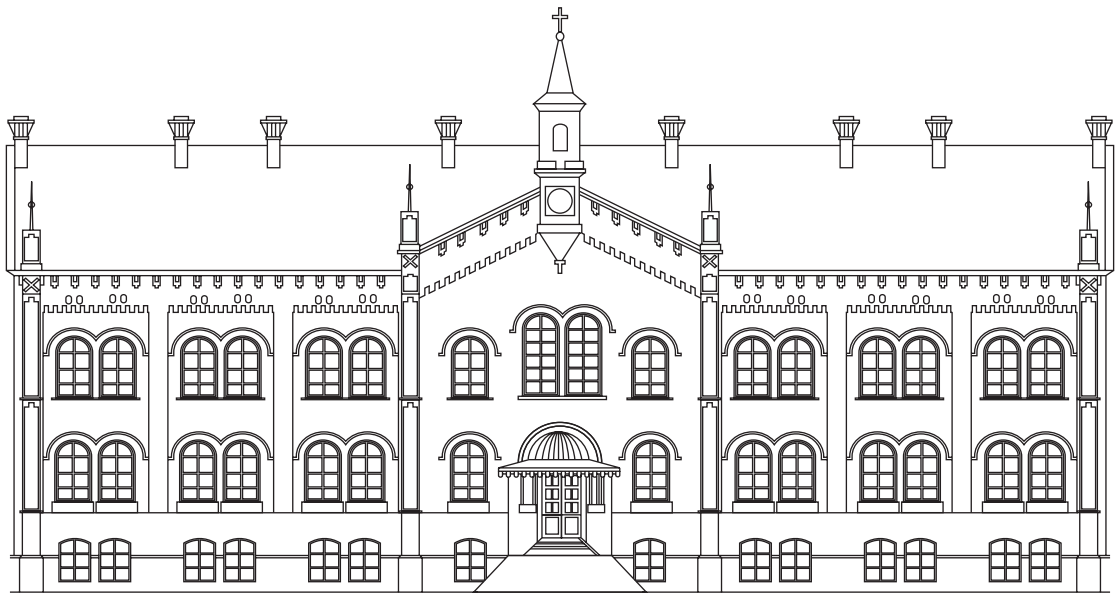
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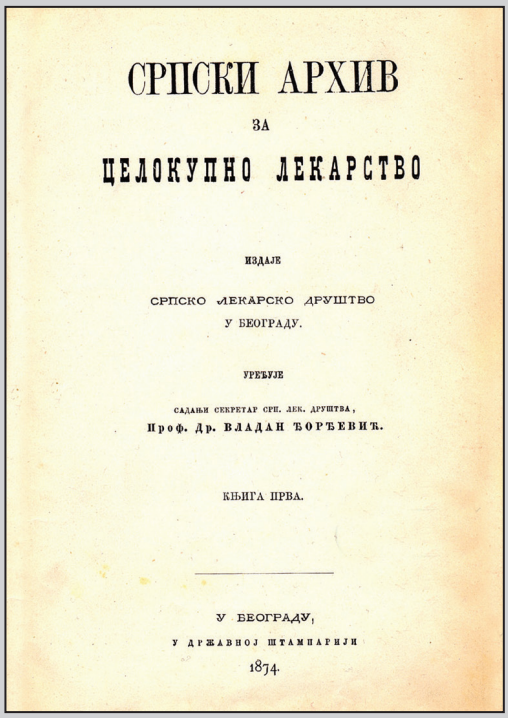
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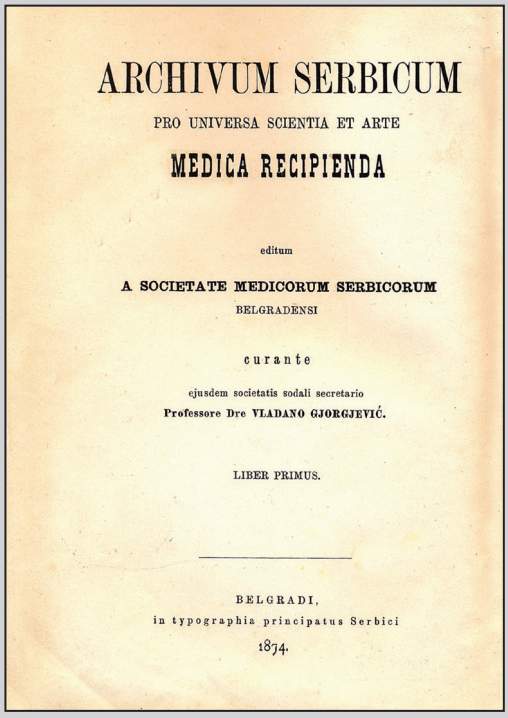
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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Vitamin D receptor gene expression in HPV-negative oral carcinomas – limited prognostic value within a complex molecular network

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SUMMARY

Introduction/Objective The vitamin D receptor (VDR) regulates cell proliferation, differentiation, apoptosis, and immune responses and has been proposed as a potential cancer biomarker. This study investigated VDR gene expression in oral squamous cell carcinoma (OSCC), an aggressive malignancy with unfavorable outcomes, particularly in HPV-negative tumors.

Methods VDR mRNA expression was analyzed by reverse transcription quantitative PCR in tumor samples from 109 HPV-negative OSCC patients, and relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Associations with clinicopathological variables were assessed using χ^2 and Fisher's exact tests, while survival was evaluated by Kaplan–Meier and Cox regression analyses.

Results VDR expression was not significantly associated with clinicopathological characteristics. Although patients with low VDR expression showed longer overall survival than those with high expression, the difference was not significant ($p = 0.253$). Borderline associations were observed in early survival, with the 24-month landmark analysis ($p = 0.050$) and among patients with recurrent tumors ($p = 0.051$). VDR expression was not an independent prognostic factor.

Conclusion VDR gene expression alone has limited prognostic value in HPV-negative OSCC. However, the observed borderline associations with short-term survival and among patients with recurrent tumors suggest a potential context-dependent role in disease progression that warrants further investigation.

Keywords: oral carcinoma; vitamin D receptor; VDR; gene expression

INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents the most common malignancy of the oral cavity and a significant cause of morbidity and mortality worldwide [1]. Despite advances in diagnostics and the implementation of multimodal therapeutic approaches, including surgery, radiotherapy, chemotherapy, and more recently immunotherapy [2], the prognosis remains unfavorable, with five-year overall survival rates of approximately 50–60%, largely due to frequent disease recurrence [1, 3]. Furthermore, the rising incidence among younger populations [4] and in regions of Southeastern Europe, including Serbia [5], highlights the growing public health significance of this malignancy.

The pathogenesis of OSCC is complex and multifactorial, involving an interplay of genetic and epigenetic alterations that disrupt the regulation of the cell cycle, differentiation, and apoptosis [2]. In addition to well-known etiological factors, such as smoking and alcohol consumption, there is growing interest in the influence of infections with human papillomavirus (HPV), which has led to the introduction of a distinct staging system and classification of oropharyngeal squamous cell carcinoma (OPSCC) and OSCC according to HPV status [6].

While the prognostic significance of HPV status is well established in OPSCC, in which HPV-positive tumors are associated with improved therapeutic response and better clinical outcomes, its role in OSCC remains insufficiently defined [6]. HPV-negative OSCC, which accounts for the majority of cases, is characterized by greater genomic instability, more aggressive clinical behavior, and poorer prognosis than HPV-positive tumors [7, 8]. These differences suggest distinct molecular and immunological pathways involved in tumor progression, including variations in antitumor immunity and tumor–host interactions. They also highlight the need for novel biomarkers to improve prognostic assessment and therapeutic decision-making [9, 10].

The vitamin D receptor (VDR) is a nuclear receptor that regulates the expression of a large number of genes involved in the control of cellular proliferation, differentiation, and apoptosis [11, 12]. Beyond its established role in calcium homeostasis, the active form of vitamin D (calcitriol), through VDR signaling, exerts pleiotropic effects, including modulation of

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immune responses, inflammatory processes, and tumor-related signaling pathways [13]. VDR signaling has been increasingly associated with modulation of innate and adaptive immune responses, regulation of inflammatory cytokine production, and maintenance of immune homeostasis within the tumor microenvironment, suggesting a possible role in immune-mediated processes and antitumor immunity [14].

VDR expression is regulated by genetic, epigenetic, hormonal, and environmental factors [11, 12], while calcitriol may induce its own expression through autoregulatory mechanisms [15].

Previous studies have demonstrated variable VDR expression in normal, premalignant, and malignant oral epithelium [16]. VDR showed a tendency toward increased expression in oral precancerous lesions compared to normal mucosa, whereas a relative decrease was observed in OSCC [17]. This pattern reflects dynamic changes in VDR signaling during disease progression [18, 19]. Furthermore, some studies suggest that VDR expression decreases with advancing tumor stage, implying its potential involvement in tumor progression and disease aggressiveness.

However, findings regarding VDR expression in oral cancer remain inconsistent, with some authors reporting increased VDR expression in malignant and premalignant lesions, while others describe reduced expression in tumor tissues. These discrepancies may reflect tumor heterogeneity, methodological differences, and variations in local vitamin D metabolism and VDR signaling within the tumor microenvironment [20]. Activation of oncogenic pathways, such as Ras signaling, and alterations in vitamin D metabolism, including increased CYP24-mediated degradation, may modulate VDR activity and its biological effects within the tumor microenvironment [21, 22].

Despite growing interest, the clinical and biological significance of VDR gene expression in HPV-negative OSCC has not yet been fully elucidated. Given the immunomodulatory and antiproliferative properties of vitamin D signaling, further investigation of VDR may contribute to a better understanding of the molecular mechanisms underlying disease progression and facilitate the identification of novel prognostic biomarkers and potential therapeutic targets. Clarification of the role of VDR signaling may also improve patient stratification and support the development of more personalized treatment approaches [9, 10, 18–20, 23].

The aim of this study was to evaluate whether VDR gene expression is associated with clinicopathological features and overall survival in patients with HPV-negative OSCC and to assess its potential prognostic value. We hypothesized that VDR expression may influence disease progression and clinical outcome through interactions within the tumor microenvironment.

METHODS

This study included 109 patients with OSCC treated surgically at the Clinic for Maxillofacial Surgery, Military Medical Academy, Belgrade. Clinical, histopathological, and follow-up data were collected from medical records. All surviving patients had a minimum follow-up period of two years. None received preoperative chemotherapy, while all underwent postoperative radiotherapy.

Tumor specimens were collected during surgery and stored at -20°C. Genomic DNA and total RNA were isolated using TRI Reagent according to the manufacturer's protocol. DNA quality was assessed by agarose gel electrophoresis, while nucleic acid concentration and purity were measured spectrophotometrically.

HPV status was determined using the HPV High Risk Screen Real-TM Quant kit targeting high- and intermediate-risk HPV types. All tumors were HPV-negative.

VDR mRNA expression was analyzed by reverse transcription quantitative PCR (RT-qPCR). Total RNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit. Amplification was performed using TaqMan assays on a 7500 Real-Time PCR System. GAPDH was selected as the reference gene following stability assessment with BestKeeper and NormFinder software. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ and Pfaffl methods. All analyses were performed in triplicate.

Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) and JMP v17 (JMP Statistical Discovery LLC, Cary, NC, USA). Associations between categorical variables were assessed using χ^2 or Fisher's exact tests. Optimal expression cutoff values were determined using Cutoff Finder [24], enabling classification into low- and high-expression groups. Survival was analyzed using Kaplan–Meier curves and log-rank tests, while hazard ratios (HRs) were calculated using Cox proportional hazards models. Statistical significance was defined as $p < 0.05$.

RESULTS

The study population consisted predominantly of males (74%), with 61% of patients younger than 58 years. Smoking was reported by 75% of participants, whereas most reported low alcohol consumption (71%). Advanced-stage tumors (stage III–IV) accounted for 71% of cases, while nodal metastases and disease recurrence were observed in 64% and 58% of patients, respectively (Table 1).

No significant association was found between VDR expression and demographic variables. Although high VDR expression was more frequent among males, the difference was not statistically significant ($p = 0.191$). Similarly, age, smoking status, and alcohol consumption showed no significant relationship with VDR expression.

Analysis of histopathological characteristics revealed no significant association between VDR expression and histological grade or nuclear grade. A non-significant tendency toward higher expression in well-differentiated tumors was observed. Likewise, no statistically significant relationship was found between VDR expression and tumor stage, tumor size, nodal status, or recurrence (Table 1).

Table 1. Association of the vitamin D receptor (VDR) gene expression with demographic and clinicopathological features of oral squamous cell carcinoma patients' tumor samples

Variable	Total N = 109 (%)		VDR expression	
			Low	High
Sex	Male	81 (74)	34	47
	Female	28 (26)	16	12
	p		0.191	
Age (≥ 58)	<	67 (61)	31	36
	>	42 (39)	19	23
	p		1.000	
Smoking	Never	27 (25)	11	16
	Ever	82 (75)	39	43
	p		0.657	
Alcohol	Low	77 (71)	37	40
	High	32 (29)	13	19
	p		0.531	
Histological grade	H1	70 (64)	29	41
	H2/3	39 (36)	21	18
	p		0.234	
Nuclear grade	N1	59 (54)	24	35
	N2/3	50 (46)	26	24
	p		0.254	
Tumor stage	S I	5 (5)	4	1
	S II	26 (24)	10	16
	S III	59 (54)	29	30
	S IV	19 (17)	7	12
	p		0.284	
Tumor stage 1/2 *vs.* 3/4	1/2	31 (28)	14	17
	3/4	78 (72)	36	42
	p		1.000	
Tumor size	T1	46 (42)	20	26
	T2	25 (23)	14	11
	T3	28 (26)	13	15
	T4	10 (9)	3	7
	p		0.540	
Tumor size 1/2 *vs.* 3/4	1/2	71 (65)	34	37
	3/4	38 (35)	16	22
	p		0.687	
Nodal metastases	N-	39 (36)	17	22
	N+	70 (64)	33	37
	p		0.841	
Recurrence	No	46 (42)	22	24
	Yes	63 (58)	28	35
	p		0.846	

Table 2. Hazard risk analysis of prognostic factors in relation to overall survival (OS) according to Cox regression analysis in oral squamous cell carcinoma patients

Cox regression analysis	Variables	OS	
		HR [95% CI]	p
Univariate analysis	Sex	0.615 (0.325–1.162)	0.134
	Age (≥ median)	0.638 (0.365–1.116)	0.115
	Smoking	1.785 (0.902–3.532)	0.096
	Alcohol	1.447 (1.115–1.878)	**0.005**
	Histological grade 1 *vs.* 2/3	1.232 (0.947–1.603)	0.121
	Nuclear grade 1 *vs.* 2/3	1.210 (0.933–1.569)	0.152
	Tumor stage 1/2 *vs.* 3/4	1.581 (1.214–2.058)	**0.001**
	Tumor size 1/2 *vs.* 3/4	1.281 (1.078–1.524)	**0.005**
	Nodal metastases	3.009 (1.559–5.807)	**0.001**
	Recurrence status	11.436 (5.226–25.026)	**< 0.0001**
Multivariate analysis	Recurrence status	11.436 (5.26–25.026)	**< 0.0001**

Univariate Cox analysis identified alcohol consumption, advanced tumor stage, larger tumor size, nodal metastases, and recurrence as factors associated with poorer overall survival. Recurrence demonstrated the strongest effect on mortality risk (HR = 11.436, p = 0.0001). In multivariate analysis, recurrence remained the only independent prognostic factor (Table 2).

Patients with low VDR expression exhibited longer mean and median overall survival (58.6 and 42 months, respectively) than those with high expression (46.5 and 29 months). However, the difference was not statistically significant (p = 0.253) (Figure 1a). Consistent findings were obtained in Cox regression analyses, where VDR expression failed to emerge as an independent predictor of survival.

When survival was evaluated using a 24-month landmark analysis, the difference reached borderline statistical significance (p = 0.050) (Figure 1b). Stratification according to tumor stage or nodal status revealed no significant survival differences between expression groups. However, among patients with recurrent disease, elevated VDR expression showed a borderline association with poorer survival (p = 0.051), whereas no such relationship was observed in patients without recurrence (Figure 2).

Overall, VDR expression showed no significant association with clinicopathological characteristics or long-term survival. Nevertheless, the borderline findings observed in landmark and recurrence-stratified analyses suggest a potential role in early disease dynamics.

DISCUSSION

The present study demonstrated that VDR gene expression is not significantly associated with key clinicopathological parameters or overall survival in patients with HPV-negative OSCC. Although patients with higher VDR expression showed a trend toward shorter survival, the difference did not reach statistical significance, suggesting that VDR expression alone has limited prognostic value in this cohort. However, borderline significance observed in the 24-month cutoff analysis and among patients with recurrent disease suggests a time-dependent prognostic effect of VDR expression in OSCC, where VDR-related mechanisms may influence early disease dynamics rather than long-term survival and clinical outcomes. Specifically, VDR expression may influence early disease progression, potentially affecting initial tumor behavior and antitumor immune responses, treatment sensitivity, or early

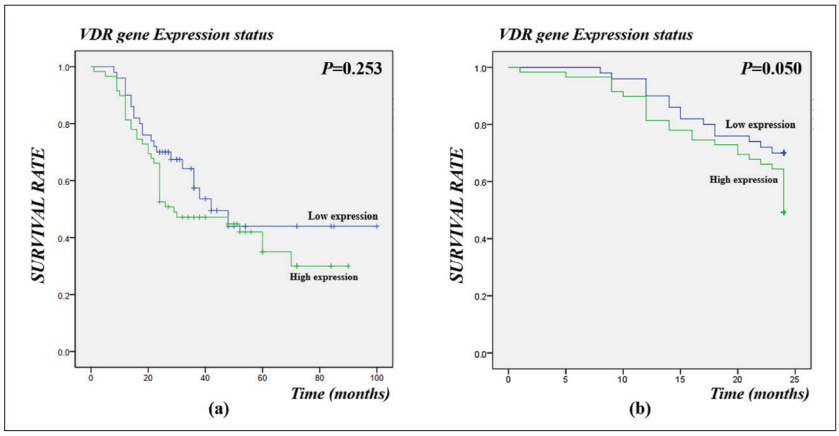


Figure 1. Kaplan–Meier survival curves with log-rank test according to dichotomized vitamin D receptor (VDR) gene expression (high *vs*. low) in the HPV-negative oral squamous cell carcinoma cohort: (a) overall survival; (b) two-year overall survival

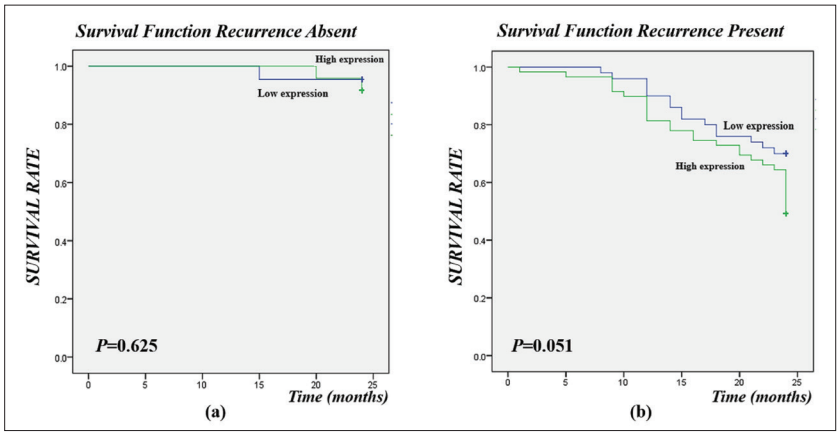


Figure 2. Kaplan–Meier survival curves according to dichotomized vitamin D receptor (VDR) gene expression (high *vs*. low) stratified by recurrence status in the HPV-negative OSCC cohort: (a) patients without recurrence; (b) patients with recurrence

recurrence dynamics. Although no significant survival differences were observed after stratification by tumor stage or nodal status, elevated VDR expression showed a borderline association with poorer survival in patients with recurrent disease, but not in those without recurrence. These findings suggest that VDR-related mechanisms may be more relevant in biologically aggressive or therapy-resistant tumors than in relation to anatomical disease extent, highlighting the complex and context-dependent role of VDR signaling in tumor biology [9, 18, 19, 20].

Biologically, this observation is plausible given the multifunctional role of the VDR in cancer biology. VDR signaling has been associated with antiproliferative, prodifferentiation, anti-inflammatory, and immunomodulatory effects in multiple malignancies, including head and neck cancers [25]. Experimental studies suggest that activation of the vitamin D_3/VDR axis may inhibit tumor cell proliferation, reduce epithelial–mesenchymal transition, modulate cytokine production, and influence antitumor immune responses [14]. Nevertheless, the consequences of VDR signaling appear to depend on tumor context, disease stage, and interactions within the tumor microenvironment. Although vitamin D/VDR signaling is generally considered protective, higher VDR expression in the present cohort was associated with a trend toward poorer survival during the earlier follow-up period. Similarly, several studies have suggested that elevated VDR expression in certain tumor settings may reflect compensatory, dysregulated, or non-functional signaling rather than effective antitumor activity. Paradoxically, in specific tumor contexts, VDR may promote pro-oncogenic phenotypes by creating immunosuppressive microenvironments or sustaining oxidative stress [14]. Therefore, elevated VDR expression may not necessarily reflect effective antitumor signaling, but could instead represent a compensatory or dysfunctional response within biologically aggressive tumors. The apparent short-term effect of VDR expression may diminish over time as clinical outcomes become increasingly influenced by stronger determinants of tumor progression, including recurrence, genetic instability, therapy resistance, and other molecular alterations. Alternatively, VDR-related effects may be restricted to specific patient subgroups or disease stages and may have been difficult to detect because of limited statistical power.

Another possible explanation is that VDR expression may be more relevant during the early stages of disease progression, reflecting tumor differentiation or initial biological behavior. As the disease advances, stronger drivers of tumor progression may predominate, diminishing the long-term prognostic impact of VDR expression. This may explain why the borderline association observed in the 24-month landmark analysis was not maintained throughout follow-up. The stronger association observed in patients with recurrent disease further suggests a potential role for VDR-related pathways in recurrence biology or treatment resistance. A critical aspect in interpreting these results is the distinction between quantitative gene expression and functional receptor activity. Importantly, VDR mRNA expression does not necessarily reflect functional receptor activity, which may be influenced by post-transcriptional regulation, altered receptor function, impaired interactions with co-regulatory proteins, such as retinoid X receptor, insufficient availability of its ligand, calcitriol, or dysregulated vitamin D metabolism [11, 12]. Consequently, increased VDR expression may represent compensatory or dysfunctional signaling rather than an indicator of effective tumor suppression [26]. This phenomenon, often referred to as "non-functional expression," may partly explain the absence of an expected correlation between VDR levels and clinical outcomes [20]. Further studies incorporating functional analyses and larger

cohorts are needed to clarify the biological and clinical significance of VDR signaling in OSCC.

Furthermore, the role of VDR in OSCC appears to be dynamic and stage-dependent. Previous studies [16, 17] have demonstrated a characteristic pattern in which VDR expression is increased in premalignant lesions and subsequently reduced in invasive carcinomas. This observation can be interpreted within the framework of clonal selection, whereby tumor cells that become less dependent on vitamin D-mediated growth regulation gain a selective advantage during disease progression. Given that the majority of tumors in the present cohort were at advanced stages (III–IV), it is plausible that VDR signaling was already functionally diminished, thereby limiting its impact on tumor behavior and patient prognosis. In such settings, VDR may no longer act as a regulator of proliferation and differentiation but instead reflect a residual or epiphenomenal feature of tumor evolution [20].

Another important consideration is that VDR operates within a broader vitamin D signaling axis, which includes key metabolic enzymes such as CYP27B1 and CYP24A1. While CYP27B1 is responsible for the activation of vitamin D, CYP24A1 mediates its degradation [27]. Overexpression of CYP24A1, frequently reported in aggressive tumors, may lead to reduced local concentrations of active vitamin D, effectively attenuating VDR signaling regardless of receptor expression levels [21]. This suggests that evaluating VDR expression alone may be insufficient and that a more comprehensive assessment of the vitamin D pathway, including key metabolic enzymes, may be necessary to fully capture the biological significance of vitamin D signaling in OSCC.

The tumor microenvironment represents an additional layer of regulation that may significantly influence VDR expression and function. Inflammatory cytokines, oxidative stress, and immune cell infiltration are known to modulate both VDR expression and vitamin D metabolism, while VDR signaling itself regulates cytokine production and immune responses. Notably, tumor and immune cells can locally synthesize or degrade vitamin D metabolites, establishing autocrine and paracrine signaling loops independent of systemic vitamin D levels [28]. This localized regulation may help explain the lack of correlation between VDR expression and clinicopathological parameters observed in this study, as key regulatory processes occur within the tumor microenvironment and may not be captured by measurements of gene expression in bulk tumor cells [20, 28]. Given the known immunomodulatory effects of vitamin D signaling, it is also plausible that VDR activity influences immune dynamics within the tumor microenvironment in a manner not detectable through isolated mRNA quantification alone.

Epigenetic mechanisms further contribute to the complexity of VDR regulation. Promoter methylation, histone modifications, and microRNA-mediated repression can all influence VDR expression and activity. These processes may result in discordance between mRNA levels and functional protein activity, thereby limiting the interpretability of transcription-based analyses [29]. In addition, alterations in chromatin accessibility may impair VDR binding to vitamin D response elements, further reducing its transcriptional efficacy [12]. Consequently, the lack of prognostic significance observed in this study may reflect underlying epigenetic dysregulation rather than the absence of biological relevance [26, 29]. Such mechanisms may be particularly relevant in HPV-negative OSCC, which is characterized by substantial molecular heterogeneity and genomic instability.

The exclusive inclusion of HPV-negative tumors in this study represents both a strength and a limitation. HPV-negative OSCC is characterized by greater genomic instability, a higher mutational burden, and frequent activation of oncogenic pathways such as Ras and PI3K/Akt [8]. Importantly, activation of these pathways has been shown to interfere with VDR-mediated transcriptional responses, thereby diminishing the biological effects of vitamin D signaling [7, 22]. This may provide an additional explanation for the lack of association between VDR expression and clinical outcomes in the present cohort, as dominant oncogenic drivers may override the regulatory influence of VDR. At the same time, restriction to HPV-negative tumors reduced biological heterogeneity associated with viral oncogenesis and enabled a more homogeneous evaluation of VDR expression within this clinically aggressive OSCC subgroup.

Despite the absence of significant findings, the present study provides important insights into the limitations of single-marker approaches in cancer prognostication. Future studies should therefore focus on integrative models incorporating multiple biomarkers, including CYP24A1, CYP27B1, and immune checkpoint molecules such as PD-1/PD-L1, in order to better capture the biological heterogeneity of OSCC [30]. The present findings also support the need for time-dependent survival analyses and functional studies evaluating not only receptor expression but also downstream signaling activity and tumor-specific vitamin D metabolism.

Several limitations of this study should be acknowledged. The sample size, although adequate for exploratory analysis, may limit the statistical power to detect subtle associations. Additionally, the analysis was restricted to mRNA expression levels without assessment of protein expression or receptor functionality. The lack of data on serum vitamin D levels and the absence of evaluation of key metabolic enzymes further constrain the interpretation of the results. Moreover, the number of survival events and the predominance of advanced-stage tumors may have influenced the ability to detect weaker prognostic effects. Future research should adopt a multidimensional approach combining transcriptomic, proteomic, and epigenetic analyses, as well as longitudinal monitoring of molecular changes during disease progression.

CONCLUSION

While VDR gene expression does not appear to have independent prognostic value in HPV-negative OSCC when assessed at the mRNA level, its role in tumor biology remains significant within a broader regulatory framework. The

findings underscore the complexity of vitamin D signaling in cancer and highlight the need for integrative approaches to fully elucidate its clinical relevance. Although the overall survival analysis did not demonstrate statistical significance, the observed borderline significance in the analysis at the 24-month time point suggests that VDR-related mechanisms may still contribute to early disease dynamics in OSCC. A deeper understanding of these mechanisms may ultimately contribute to the development of personalized therapeutic strategies and the identification of novel molecular targets in the management of oral cancer.

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Ethics: The study was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia (Approval No. 52/2024).

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Експресија гена за рецептор витамина *D* код *HPV*-негативних оралних карцинома – ограничени прогностички значај у оквиру сложене молекуларне мреже

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САЖЕТАК

Увод/Циљ Рецептор витамина *D* (*VDR*) учествује у регулацији ћелијске пролиферације, диференцијације, апоптозе и имунског одговора и представља потенцијални биомаркер у онкологији. Циљ ове студије био је да се испита експресија гена *VDR* у оралном сквамоцелуларном карциному (*OSCC*), агресивном малигнитету са неповољним исходом, посебно код *HPV*-негативних тумора.

Методе Експресија *VDR mRNA* анализирана је методом *RT-qPCR* у туморским узорцима 109 болесника са *HPV*-негативним *OSCC*, а релативна експресија израчуната је применом методе $2^{-\Delta\Delta Ct}$. Повезаност са клиничкопатолошким параметрима процењена је χ^2 тестом и Фишеровим егзактним тестом, док је преживљавање анализирано Каплан–Мајеровом методом и Коксовом регресионом анализом.

Резултати Није утврђена статистички значајна повезаност експресије *VDR* са клиничкопатолошким карактеристикама. Иако су болесници са ниском експресијом *VDR* имали дуже укупно преживљавање у односу на болеснике са високом експресијом, разлика није достигла статистичку значајност ($p = 0,253$). Граничне статистичке значајности уочене су у анализи раног преживљавања са пресеком на 24 месеца ($p = 0,050$), као и код болесника са рецидивом тумора ($p = 0,051$). Експресија *VDR* није представљала независан прогностички фактор.

Закључак Експресија гена *VDR* у ткиву оралних карцинома сама по себи показала је ограничен прогностички значај код *HPV*-негативних *OSCC*. Ипак, уочене граничне вредности статистичке значајности у анализи раног преживљавања и преживљавања болесника са рецидивом тумора указују на могућу улогу *VDR* у прогресији болести која зависи од контекста, што захтева даља истраживања.

Кључне речи: орални карцином; рецептор витамина *D*; експресија гена

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Left atrial appendage occlusion devices – a contemporary approach to stroke prevention with a presentation of results in Serbia from 2014 to 2025

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SUMMARY
Introduction/Objective Atrial fibrillation is the most common sustained cardiac arrhythmia and one of the most significant independent risk factors for ischemic stroke and systemic thromboembolism. In patients with non-valvular atrial fibrillation, the left atrial appendage (LAA) is the predominant site of intracardiac thrombus formation. This has led to the development of mechanical elimination of the thrombogenic substrate, i.e., percutaneous LAA occlusion (LAAO), as an alternative to long-term oral anticoagulant therapy in patients with contraindications to anticoagulation.
Methods The first LAAO device in Serbia was implanted on April 24, 2014, at BASICS+8, University Clinical Center of Serbia, by Prof. Dr. Milan A. Nedeljković and Prof. Dr. Branko Beleslin. LAAO device implantation is currently performed at four centers: University Clinical Center of Serbia, Dedinje Institute for Cardiovascular Diseases, Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica, and Bežanijska kosa University Clinical Center. Before LAAO implantation, either transesophageal echocardiography (TEE) or computed tomography is performed to determine the morphology of the LAA and confirm the absence of thrombus, which represents a contraindication to the procedure. The intervention is performed via femoral venous access under short-term analgosedation or general anesthesia, depending on the practice of the center, with fluoroscopic guidance and TEE monitoring. The key step is transseptal puncture to gain access to the left atrium. TEE guidance is used to ensure precise puncture of the inferoposterior portion of the interatrial septum and facilitate positioning of the delivery system at the LAA ostium.
Results Between 2014 and 2025, a total of 30 LAAO devices were implanted at four centers in Serbia. With support from the Republic Health Insurance Fund, two devices were implanted at University Clinical Center of Serbia in 2014, followed by one additional device in 2015. No data on the use of this method in angiography suites in Serbia were available for the period from 2016 to 2022. In 2023, a total of six devices were implanted (four at Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica and two at Dedinje Institute for Cardiovascular Diseases). In 2024, a total of 10 devices were implanted (six at Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica, one at Dedinje Institute for Cardiovascular Diseases, and three at Bežanijska kosa University Clinical Center). In 2025, a total of 11 devices were implanted (five at Dedinje Institute for Cardiovascular Diseases and six at the Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica).
Conclusion The use of percutaneous LAAO in Serbia demonstrates gradual development, from the initial sporadic application of the method at a single center to its expansion to multiple tertiary cardiology centers. Further development of LAAO in Serbia should be accompanied by the establishment of a national registry and standardized data collection.
Keywords: atrial fibrillation; left atrial appendage; percutaneous left atrial appendage occlusion; oral anticoagulant therapy; direct oral anticoagulant therapy; stroke

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and one of the most significant independent risk factors for ischemic stroke and systemic thromboembolism. Thromboembolic complications of AF are associated with substantial morbidity, mortality, and long-term disability, making stroke prevention one of the primary goals of treatment in patients with AF. In patients with non-valvular AF, the left atrial appendage (LAA) is the predominant site of intracardiac thrombus formation. This recognition has led to the development of the concept of mechanical elimination of the thrombogenic substrate, namely percutaneous LAA occlusion (LAAO), as an alternative to long-term oral anticoagulant therapy (OAC). Oral anticoagulation, initially with vitamin K antagonists and subsequently with direct oral anticoagulants (DOACs), represents the cornerstone of thromboembolic event prevention in patients with AF. Although effective, long-term anticoagulation carries a risk of bleeding, may be challenging in patients with previous hemorrhagic events or other contraindications, and represents a significant treatment burden for some patients. These limitations have prompted the development of interventional approaches aimed at providing adequate thromboembolic protection while reducing the long-term risk of bleeding. The development of LAAO devices began with the demonstration of the feasibility and efficacy of this strategy compared with warfarin. Early randomized trials, particularly PROTECT AF [1, 2] and PREVAIL

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Table 1. Left atrial appendage occluders in Serbia, 2014–2025, according to available data from the Working Group for Interventional Cardiology of the Cardiology Society of Serbia, BASICS+ (Belgrade Summit of Interventional Cardiology, Cardiovascular Therapy and Imaging)

Year	Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica	University Clinical Centre of Serbia	Dedinje Institute for Cardiovascular Diseases	Bežanijska kosa University Clinical Center	Total
2014	N/A	2*	N/A	N/A	2
2015	N/A	1*	N/A	N/A	1
2016	N/A	N/A	N/A	N/A	0
2017	N/A	N/A	N/A	N/A	0
2018	N/A	N/A	N/A	N/A	0
2019	N/A	N/A	N/A	N/A	0
2020	N/A	N/A	N/A	N/A	0
2021	N/A	N/A	N/A	N/A	0
2022	N/A	N/A	N/A	N/A	0
2023	4**	N/A	2**	N/A	6
2024	6**	N/A	1**	3**	10
2025	6**	N/A	5**	N/A	11
Total	16	3	8	3	30

*Procedure funded by the Republic Health Insurance Fund;
**Procedures performed using occluders procured through hospital tenders

[3], provided the basis for the acceptance of LAAO as a potential alternative to oral anticoagulation. At the same time, experience from the initial studies highlighted the importance of procedural risk and led to the development of new generations of devices, with the aim of achieving better adaptation to the diverse anatomical characteristics of the LAA, more complete sealing, and a reduction in complications. With the advent of direct oral anticoagulants (DOACs), the context in which the role of LAAO is evaluated has also changed. PRAGUE-17 [4] represented an important milestone, as LAAO was directly compared with DOAC therapy in patients at high thromboembolic and bleeding risk. Furthermore, studies such as Amulet IDE [5] and PINNACLE FLX [6] enabled comparisons between different devices and assessment of the safety and efficacy of contemporary generations of occluders. The latest data from large randomized trials, including OPTION [7] and CHAMPION-AF [8], have further expanded the potential indications for LAAO, while the results of the CLOSURE-AF study [9] simultaneously indicate that this strategy cannot be considered a universal replacement for contemporary medical therapy. While international landmark trials continuously expand the clinical landscape and indications for LAAO, evaluating how these guidelines translate into real-world practice within smaller, resource-constrained healthcare systems remains essential.

Thus, the development of LAAO represents an example of the continuous evolution of interventional therapy – from the initial proof of concept and comparison with warfarin, through improvements in procedural safety and the development of new devices, to direct comparisons with contemporary anticoagulant therapy. Understanding the results of key randomized trials is essential for appropriately defining the role of LAAO in contemporary stroke prevention in patients with AF. The aim of this paper is to present, through a review and critical analysis of the most important randomized clinical trials, the evolution of evidence regarding the efficacy and safety of LAAO, the development of the devices themselves, their comparison with OAC, and the current role of LAAO in the strategy for preventing thromboembolic complications in patients with non-valvular AF, as well as the results of LAAO implantation in catheterization laboratories in Serbia from 2014 to 2025.

METHODS

In Serbia, the first LAAO device was implanted on April 24, 2014, using the BASICS+8 device at University Clinical Center of Serbia by Prof. Dr. Milan A. Nedeljković and Prof. Dr. Branko Beleslin (Table 1) [10]. LAAO device implantation is currently performed at four centers: the University Clinical Center of Serbia, Dedinje Institute for Cardiovascular Diseases, Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica and Bežanijska kosa University Clinical Center. Prior to the decision to implant an LAAO device, either transesophageal echocardiography (TEE) or computed tomography (CT) is performed to determine the morphology of the LAA (chicken wing, broccoli, windsock, or cactus morphology) and to confirm the absence of thrombus within the appendage, as the presence of thrombus represents a contraindication to the procedure. The size of the occluder is determined based on the measurements obtained, primarily the depth of the appendage and the diameter of the appendage ostium, which are assessed by TEE in multiple views (0°, 45°, 90°, and 135°), as well as by CT image reconstruction.

The procedure is performed via femoral venous access under short-term conscious sedation or general anesthesia, depending on the center's practice, with fluoroscopic guidance and TEE monitoring. The key step is transseptal puncture to gain access to the LAA. This is performed under TEE guidance to ensure precise puncture of the inferoposterior portion of the interatrial septum and facilitate advancement of the delivery system toward the appendage ostium. After reconfirming the dimensions of the appendage by TEE, an appropriately sized occluder is selected and positioned within the LAA. Following occluder

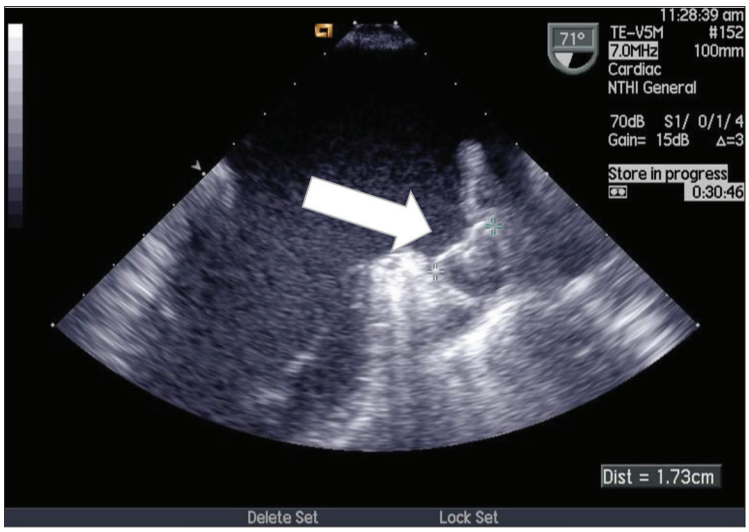


Figure 1. The first Watchman™ occluder (Watchman, Boston, MA, USA) implanted in a catheterization laboratory in Serbia on April 24, 2014

implantation and before its definitive release, several key parameters are assessed, including appropriate device position and size, as determined by the degree of compression, device stability, and the absence of peri-device leak.

Potential periprocedural complications include pericardial effusion, occluder embolization, and procedure-related stroke [11, 12].

In catheterization laboratories in Serbia, the Watchman™ (Figure 1) and Watchman FLX™ occluders (Watchman, Boston, MA, USA) and the Amplatzer™ AMULET™ occluder (Abbott Laboratories, Abbott Park, IL, USA) have been used.

Ethics: This study was conducted in accordance with the ethical standards of the institutional ethics committee.

RESULTS

Between 2014 and 2025, a total of 30 LAA occluders were implanted at four centers in Serbia. In 2014, with the support of the Republic Health Insurance Fund, two occluders were implanted at University Clinical Center of Serbia. The Republic Health Insurance Fund covered one additional procedure in 2015, which was also performed at University Clinical Center of Serbia.

Between 2016 and 2022, no data on the use of this method in catheterization laboratories in Serbia are available according to the data published by the Working Group for Interventional Cardiology of the Cardiology Society of Serbia in the Belgrade Summit of Interventional Cardiology, Cardiovascular Therapy and Imaging (BASICS+) program.

In 2023, a total of six procedures were performed (four at the Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica and two at Dedinje Institute for Cardiovascular Diseases) according to the available data from the Working Group for Interventional Cardiology of the Cardiology Society of Serbia.

In 2024, a total of 10 occluders were implanted (six at the Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica, one at Dedinje Institute for Cardiovascular Diseases, and three at Bežanijska kosa University Clinical Center).

In 2025, a total of 11 occluders were implanted (five at Dedinje Institute for Cardiovascular Diseases and six at the Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica).

DISCUSSION

The results of this analysis indicate a gradual development of the percutaneous LAAO program in Serbia, with a particularly pronounced increase in the number of procedures during the last three years of the study period. According to the available data, a total of 30 LAA occluders were implanted at four centers in Serbia between 2014 and 2025. Following the initial procedures performed in 2014 and 2015 [10], the number of interventions increased substantially from 2023 onward, with six procedures recorded that year, while the highest number, a total of 11 was recorded in 2025. This trend indicates the gradual expansion of the use of this method in Serbia [13, 14].

The first procedures were recorded in 2014, when two occluders were implanted at the University Clinical Center of Serbia with financial support from the Republic Health Insurance Fund, followed by one additional procedure in 2015. This period marked the beginning of LAAO implementation in Serbia, at a time when the method was already the subject of intensive clinical investigation and development. LAAO was developed with the aim of reducing the risk of thromboembolic events in selected patients with AF, particularly when long-term OAC is unsuitable or associated with an unacceptable risk. Current European guidelines recognize LAAO as part of stroke prevention strategies in carefully selected patients, with an individualized assessment of its potential benefits and risks [15, 16].

In the study by Pison et al. [17], published in 2015, LAAO was still described as a relatively new and insufficiently widespread procedure. Most European centers performing the procedure had carried out ≤ 30 interventions throughout 2014, with the most common indication being a high thromboembolic risk in the presence of a contraindication to OAC.

The distribution of procedures across centers indicates that the use of LAAO in Serbia is concentrated in several tertiary cardiology institutions. In 2023, four procedures were performed at the Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica and two at Dedinje Institute for Cardiovascular Diseases. In 2024, six procedures were performed at the Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica, one at Dedinje Institute for Cardiovascular Diseases, and three

at Bežanijska kosa University Clinical Center. In 2025, five procedures were performed at Dedinje Institute for Cardiovascular Diseases and six procedures were performed at the Institute for Cardiovascular Diseases of Vojvodina – Sremska Kamenica. Such concentration may be expected during the early development of a new interventional technique, as it facilitates the accumulation of experience, establishment of multidisciplinary teams, and concentration of technical and diagnostic resources. European data also indicate substantial variation in procedural volume between centers, with some centers performing very few interventions annually.

In an international consensus practical guide, Potpara et al. [18] emphasized that the contemporary development of LAAO has been associated with improvements in procedural safety and its increasingly frequent use in patients with AF for whom long-term anticoagulation is not an appropriate option.

The stark contrast between the expanding indications for LAAO in international guidelines and the modest real-world volume of 30 cases over more than a decade in Serbia highlights significant structural hurdles. The complete absence of recorded procedures between 2016 and 2022 reflects a temporary nationwide standstill of the program, primarily driven by strict economic constraints, inconsistent reimbursement pathways by the National Health Insurance Fund, and logistical issues with hospital tenders, rather than a lack of operator expertise.

This long-term stagnation emphasizes that transitioning from a sporadic, center-specific approach to a structured national registry is a mandatory next step. Establishing such a framework will be vital to provide the standardized clinical data necessary to justify broader and more consistent financial coverage for this stroke-prevention strategy in the future.

CONCLUSION

The use of percutaneous LAAO in Serbia has demonstrated gradual development, from the initial sporadic application of the technique at a single center to its expansion to several tertiary cardiology centers. The first procedures, described in the study by Nedeljković and colleagues, marked the beginning of the implementation of this method in Serbia, while available data from the period 2023–2025 indicate an increase in the number of procedures and expansion of the LAAO program to multiple centers. This trend is consistent with the development of LAAO in Europe and its increasingly important role in the prevention of thromboembolic events in carefully selected patients with AF for whom long-term OAC is not an appropriate option.

Further development of LAAO in Serbia should be accompanied by the establishment of a national registry and standardized data collection, enabling assessment of procedural availability and quality, comparison of outcomes between centers, and appropriate positioning of LAAO within contemporary treatment strategies for patients with AF.

Conflict of interest: None declared.

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Оклудери аурикуле леве преткоморе – савремени приступ у превенцији можданог удара са приказом резултата у Србији 2014–2025

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САЖЕТАК

Увод/Циљ Атријална фибрилација представља најчешћу трајну срчану аритмију и један од најзначајнијих независних фактора ризика за настанак исхемијског можданог удара и системске тромбоемболије. Код болесника са невалвуларном атријалном фибрилацијом, аурикула леве преткоморе (*LAA*) представља доминантно место настанка интракардијалног тромба. Ово је довело до развоја механичке елиминације тромбогеног супстрата, односно перкутане оклузије *LAA* (*LAAO*) као алтернативе дуготрајној оралној антикоагулантној терапији код болесника код којих постоји контраиндикација за њену примену.

Методе У Србији је први уређај за *LAAO* уграђен 24. априла 2014. године на *BASICS*+8 у Универзитетском клиничком центру Србије, а процедуру су извели проф. др Милан А. Недељковић и проф. др Бранко Белеслин. Имплантација оклудера обавља се у четири центра: Универзитетском клиничком центру Србије, Институту за кардиоваскуларне болести „Дедиње", Институту за кардиоваскуларне болести Војводине – Сремска Каменица и Универзитетском клиничком центру „Бежанијска коса". Пре доношења одлуке о извођењу *LAAO* раде се трансезофагеална ехокардиографија (TEE) или компјутеризована томографија како би се утврдио облик леве аурикуле и потврдило одсуство тромба у аурикули, с обзиром на то да присуство тромба представља контраиндикацију за извођење саме процедуре. Интервенција се изводи приступом преко феморалне вене, у краткотрајној аналгоседацији или општој анестезији, у зависности од праксе центра, уз флуороскопију и под контролом TEE. Основни корак представља транссептална пункција, којом се приступа левој аурикули. Она се изводи под контролом TEE како би се омогућила прецизна пункција инферопостериорног септума и олакшало усмеравање система за испоручивање ка улазу у аурикулу.

Резултати Између 2014. и 2025. године укупно је имплантирано 30 оклудера за *LAA* у четири центра у Србији. Захваљујући Републичком фонду за здравствено осигурање, 2014. године су у Универзитетском клиничком центру Србије уграђена два оклудера, а 2015. године још један. Између 2016. и 2022. године нема доступних података о примени ове методе у ангиографским салама у Србији. Године 2023. изведено је укупно шест имплантација (четири у Институту за кардиоваскуларне болести Војводине – Сремска Каменица и две у Институту за кардиоваскуларне болести „Дедиње"). Године 2024. уграђено је укупно 10 оклудера (шест у Институту за кардиоваскуларне болести Војводине – Сремска Каменица, један у Институту за кардиоваскуларне болести „Дедиње" и три у Универзитетском клиничком центру „Бежанијска коса"). Године 2025. уграђено је укупно 11 оклудера (пет у Институту за кардиоваскуларне болести „Дедиње" и шест у Институту за кардиоваскуларне болести Војводине – Сремска Каменица).

Закључак Примена перкутане *LAAO* у Србији показује постепен развој од иницијалне, спорадичне примене методе у једном центру до њеног ширења на више терцијарних кардиолошких центара. Даљи развој *LAAO* у Србији требало би да буде праћен формирањем националног регистра и стандардизованим прикупљањем података.

Кључне речи: атријална фибрилација; аурикула леве преткоморе; перкутана оклузија аурикуле леве преткоморе; орална антикоагулантна терапија; директна орална антикоагулантна терапија; мождани удар

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Outcome of COVID-19 infection in patients with kidney transplant in the first two years of the pandemic – our experience with the use of tocilizumab and monoclonal anti-SARS-CoV-2 antibodies

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SUMMARY

Introduction/**Objective** Kidney transplant recipients were at higher risk for COVID-19–associated complications and mortality. The goal was to examine the frequency and outcomes of COVID-19 infection among patients under our center's control, as well as the effects of tocilizumab and anti-SARS-CoV-2 antibodies.

Methods We analyzed a cohort of 220 kidney transplant patients monitored at our center that experienced COVID-19 infection between March 2020 and March 2022.

Results During the two-year-long period, 55 patients contracted COVID-19 (25%). The average age of the infected was 48.38 years. In 24% of patients during COVID-19 infection the immunosuppressive therapy did not change. Because of pneumonia development, 33% of patients have been hospitalized. Of all infected patients, tocilizumab was used in 7% of patients, of whom three died. Anti-SARS-CoV-2 monoclonal antibodies were used in 24% of patients, no fatalities were reported. Antiviral medication (favipiravir) was used in 4% of patients, in one case fatal. Continuous renal replacement therapy / hemodialysis was applied in 5.5% of patients. Out of all infected, 14.5% of patients had a fatal outcome. The cause of death was severe acute respiratory syndrome (50%), cardiac arrest (25%), or multiorgan failure (25%).

Conclusions The therapeutic use of monoclonal antibodies was reliable, with a significant positive effect on achieving a milder clinical form and shorter duration of the disease. The main risk factors for adverse outcomes of COVID-19 infection in kidney transplant recipients were serious comorbidities and late presentation to the transplant center.

Keywords: kidney transplant; COVID-19 infection; anti-SARS-CoV-2 antibodies

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic caused by the highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had led to unprecedented challenges for healthcare systems worldwide. The pandemic rapidly spread worldwide after the first reported case of COVID-19 in Wuhan, China, in late December 2019, prompting the World Health Organization to declare it a global pandemic on 11 March 2020. Since the initial phase, substantial progress in clinical research had led to a better understanding of SARS-CoV-2, and multiple medical therapies to treat the disease emerged [1, 2, 3].

Kidney transplant recipients (KTRs) remained at higher risk for COVID-19-associated complications and mortality. Compared with matched nontransplant patients, KTRs experience a doubling of risk of COVID-19-related death, especially when considering age, body mass index, and other major comorbidities. Therefore, the identification of early interventions that can halt the progression from mild or moderate to severe COVID-19 is especially important in this vulnerable population [4, 5].

KTRs were vulnerable to infections due to their ongoing immunosuppressive therapy, impaired vaccine response, presence of other comorbidities, and, in some cases, impaired kidney-graft function. Identifying modifiable risk factors that increased the susceptibility to severe COVID-19 could help target prevention and treatment strategies, thus improving future outcomes in this population [2].

Age and comorbidities are known risk factors associated with COVID-19 severity and higher mortality in the general population and among solid organ transplant (SOT) recipients [6, 7].

Numerous therapeutic interventions have been tested to control the spread of the COVID-19 infection. However, to this date, no specific agent had been found that guarantees safe and effective control of COVID-19. Monoclonal antibodies (mAbs) such as

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casirivimab, imdevimab, bamlanivimab, etesevimab, sotrovimab, and tocilizumab have gotten emergency use authorization for patients infected with COVID-19. These mAbs targeted the proteins of SARS-CoV-2 that helped the virus bind to human cells. Additionally, some of them also reduced the action of cytokines, thereby minimizing the inflammatory reactions considered to be responsible for COVID-19 complications [8–13].

Bamlanivimab and casirivimab-imdevimab prevented viral entry into host cells by blocking the binding of the spike protein to the cell surface angiotensin-converting enzyme 2 receptor. These drugs, combined with immunosuppression reduction for outpatient treatment of mild to moderate COVID-19 in KTRs, were safe and had good outcomes [4]. Tocilizumab represented a valuable therapeutic option for managing COVID-19 complications, particularly in patients at risk of cytokine storm syndrome. The mechanism of action of tocilizumab involves binding to interleukin-6 receptors, inhibiting pro-inflammatory responses in a variety of cells, including lymphocytes, monocytes, fibroblasts, and B-cells [10–19].

Modifications of the immunosuppressive regimen were a part of the therapeutic prescription in SOT recipients who developed COVID-19 infection. These include suspending antimetabolites and/or reducing calcineurin inhibitor dosing in asymptomatic patients and patients not requiring hospitalization. In COVID-19 infected patients needing low-flow oxygen, a dose reduction of the metabolite and/or reduction of calcineurin inhibitors or mTOR inhibitor levels might be necessary. In more severe cases, some centers applied a more significant immunosuppression reduction strategy, with temporary discontinuation of all immunosuppressive drugs, except steroids. This strategy needed to be balanced with a potential increased risk for the development of acute rejection and/or graft loss. However, other experts proposed continuation of calcineurin inhibitors (particularly cyclosporine) during advanced disease to control the inflammatory phase. In any case, the complex management of immunosuppression during the course of infection should have been discussed in a multidisciplinary approach by transplant physicians, intensive care unit doctors, and transplant infectious diseases specialists [14].

The goal of this study was to examine the frequency and outcome of the COVID-19 infection within patients controlled at our center, as well as the effect of tocilizumab and anti-SARS-CoV-2 antibodies.

METHODS

We performed a retrospective, single-center study of adult kidney transplant (KT) recipients with a positive reverse-transcriptase polymerase chain reaction (RT-PCR) test for SARS-CoV-2 or positive SARS-CoV-2 antigen test, from March 1, 2020, to February 28, 2022, at the University Clinical Center of Vojvodina. Informed consent was obtained in accordance with the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects.

At the time of data collection, 220 KT recipients were followed up at our center. All had a functional KT. We defined adult KT recipients with PCR- or AG-test confirmed SARS-CoV-2 infection. SARS-CoV-2 RT-PCR testing was performed on nasopharyngeal swab samples. ELISA measured IgM and IgG antibodies for SARS-CoV-2 receptor binding domain spike.

Demographic and clinical data, including transplant characteristics, comorbidities, immunosuppressive therapy, intensive care unit admission, treatment with mAbs/antivirals, acute kidney injury (AKI), renal replacement therapy (RRT), and all-cause mortality rates were registered. All cases were followed up for at least 60 days after diagnosis.

All data were collected from the patients and their medical records during the treatment of COVID-19 infection and subsequent treatment.

The specific COVID-19 treatment depended on the timing of the pandemic; a protocol according to the national guide for treatment was applied and was furthermore changed during the pandemic [20].

Statistical analyses were conducted using RStudio 2025.05.0+496 "Mariposa Orchid" Release. The normality of continuous variables was assessed using the Kolmogorov–Smirnov test. Continuous variables are reported as mean ± standard deviation (SD), and as a median with interquartile range (IQR; Q1–Q3); range is provided for all continuous variables. Continuous variables were compared between independent groups using the Student's t-test or Wilcoxon rank-sum test, as appropriate, while categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate. All statistical tests were two-tailed, and an α level of 0.05 was set as the significance threshold. No imputation was performed for missing data.

RESULTS

Out of 220 KTRs that are under our supervision at the University Clinical Center of Vojvodina, in the first two years of the COVID-19 pandemic, 55 (25%) patients had been infected with COVID-19. In the first year, 23 patients were infected, while in the second year, 32 patients were infected.

Baseline characteristics of KTRs with COVID-19 infection are shown in Table 1. The average age of those infected was 48.38 years old (the youngest being 26 years old, the oldest 71 years old), with the greatest percentage ranging from 41 to 65 years old (60%), followed by the age group ranging from 26 to 40 years old (27%), ending with the age group of patients over 65 years old (13%) (Figure 1).

A total of 53% of those infected received a transplant from a living-related donor, and 47% from a deceased donor. The average time from KT to COVID-19 infection was 8.13 years; 40% of patients were infected in the first five years after KT (Tables 1 and 2; Figure 2); 67% were male.

All patients were on triple immunosuppressive therapy (calcineurin inhibitor, antimetabolite, corticosteroid). In 24% of the patients who had lighter clinical manifestations of the infection and were treated in home conditions, during

Table 1. Baseline characteristics of kidney transplant recipients with COVID-19 (n = 55)

Characteristic	n = 55 (100%)
Age (years)	
Mean ± SD	48.4 ± 12.6
Median: [Q1–Q3]	Median: 47 [39–58]
Range: Min–Max	Range: 26–71
Age group, n (%)	
26–40 yrs	15 (27%)
41–65 yrs	33 (60%)
> 65 yrs	7 (13%)
Time from transplant to COVID-19 (years)	
Mean ± SD	8.1 ± 5.7
Median: [Q1–Q3]	Median: 7 [3–13]
Range: Min–Max	Range: 1–24
Creatinine before COVID-19 (μmol/L)	n = 55
Mean ± SD	144.9 ± 54.6
Median: [Q1–Q3]	Median: 122 [105–179]
Range: Min–Max	Range: 90–300
Creatinine after COVID-19 (μmol/L)	n = 47
Mean ± SD	138.7 ± 58.9
Median: [Q1–Q3]	Median: 114 [100–156]
Range: Min–Max	Range: 88–330
Antimetabolite, n (%)	
MMF	47 (85%)
Everolimus	6 (11%)
Azathioprine	2 (3.6%)
Calcineurin inhibitor, n (%)	
Tacrolimus	48 (89%)
Cyclosporine A	6 (11%)
Antimetabolite discontinued, n (%)	37 (67%)
Hypertension, n (%)	34 (62%)
Diabetes mellitus, n (%)	8 (15%)
Cardiomyopathy, n (%)	7 (13%)
Systemic lupus erythematosus, n (%)	5 (9.1%)
Late referral / respiratory insufficiency, n (%)	2 (3.6%)
Hospital admission, n (%)	16 (29%)
Renal replacement therapy (CRRT/HD), n (%)	4 (7.3%)
Casirivimab-imdevimab, n (%)	12 (22%)
Bamlanivimab, n (%)	1 (1.8%)
Tocilizumab, n (%)	4 (7.3%)
Favipiravir, n (%)	2 (3.6%)
Lethal outcome, n (%)	8 (15%)

CRRT/HD – continuous renal replacement therapy / hemodialysis

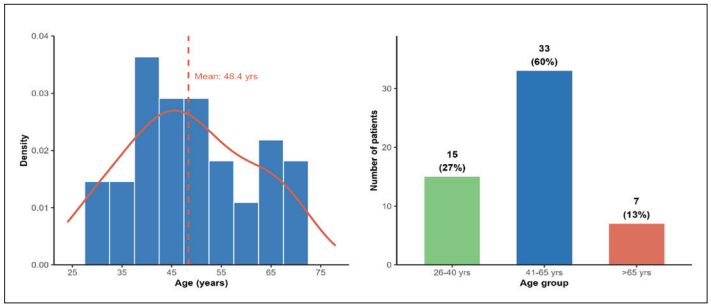


Figure 1. Age of kidney transplant recipients with COVID-19 infection

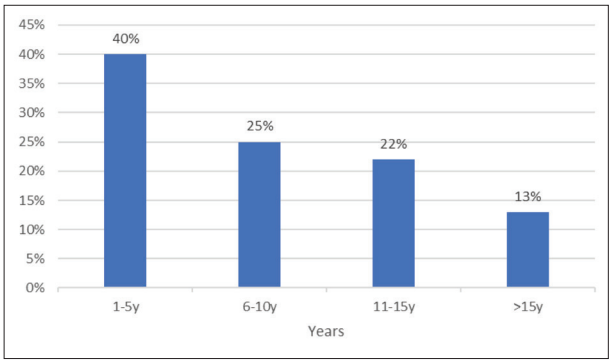


Figure 2. Time between kidney transplant and COVID-19 infection

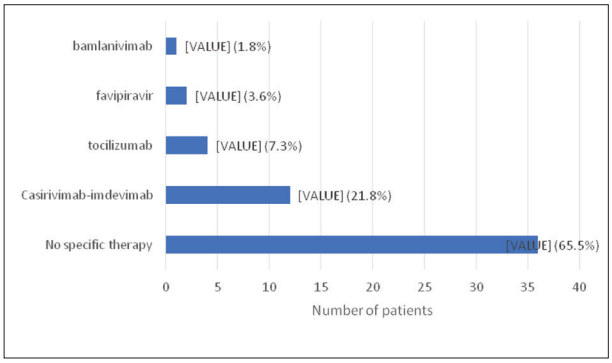


Figure 3. Specific Antiviral Therapeutic Protocols in all patients

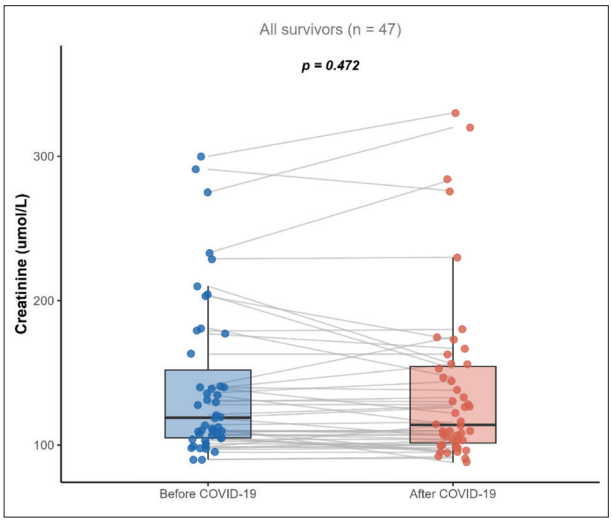


Figure 4. Kidney transplant function before and after COVID-19 in all surviving kidney transplant recipients

the COVID-19 infection, immunosuppressive therapy was not changed; in 67% of all patients, antimetabolite therapy was excluded (mycophenolate mofetil/mycophenolate sodium/azathioprine), the corticosteroid dose was increased, calcineurin inhibitor dose was decreased by 25–50%.

Considering specific antiviral therapeutic protocol out of the total number of those infected (55), 36 (65.5%) did not receive tocilizumab, an anti-SARS-CoV-2 monoclonal antibody or antiviral treatment; anti-SARS-CoV-2 monoclonal antibody (bamlanivimab or casirivimab-imdevimab) was used in 13 (23.6%) patients, from whom only two had been hospitalized with pneumonia, and in

Table 2. Comparison of survivors and deceased patients

Characteristic	Overall n = 55	Survived n = 47	Deceased n = 8	p[1]
Age (years)				< 0.001
Mean ± SD	48.4 ± 12.6	46 ± 11.9	62.6 ± 5.4	
Median: [Q1–Q3]	Median: 47 [39–58]	Median: 46 [38–53]	Median: 64.5 [59.5–66.5]	
Age group, n (%)				0.019
26–40 yrs	15 (27%)	15 (32%)	0 (0)	
41–65 yrs	33 (60%)	28 (60%)	5 (63%)	
> 65 yrs	7 (13%)	4 (8.5%)	3 (38%)	
Time from transplant to COVID-19 (years)				0.464
Mean ± SD	8.1 ± 5.7	7.8 ± 5.4	9.8 ± 7.2	
Median: [Q1–Q3]	Median: 7 [3–13]	Median: 7 [3–12]	Median: 9 [3–14.5]	
Creatinine before COVID-19 (μmol/L)				0.102
Mean ± SD	144.9 ± 54.6	140.6 ± 54.1	170.1 ± 54.4	
Median: [Q1–Q3]	Median: 122 [105–179]	Median: 119 [105–163]	Median: 166 [128.5–219]	
Antimetabolite-before COVID-19, n (%)				0.690
MMF	47 (85%)	39 (83%)	8 (100%)	
Everolimus	6 (11%)	6 (13%)	0 (0)	
Azathioprine	2 (3.6%)	2 (4.3%)	0 (0)	
Calcineurin inhibitor-before COVID-19, n (%)				0.213
Tacrolimus	48 (89%)	42 (91%)	6 (75%)	
Cyclosporine A	6 (11%)	4 (8.7%)	2 (25%)	
Antimetabolite discontinued, n (%)	37 (67%)	31 (66%)	6 (75%)	> 0.999
Hypertension, n (%)	34 (62%)	26 (55%)	8 (100%)	0.018
Diabetes mellitus, n (%)	8 (15%)	6 (13%)	2 (25%)	0.329
Cardiomyopathy, n (%)	7 (13%)	2 (4.3%)	5 (63%)	< 0.001
Systemic lupus erythematosus, n (%)	5 (9.1%)	4 (8.5%)	1 (13%)	0.559
Late referral / respiratory insufficiency, n (%)	2 (3.6%)	0 (0)	2 (25%)	0.019
Hospital admission, n (%)	16 (29%)	8 (17%)	8 (100%)	< 0.001
Renal replacement therapy (CRRT/HD), n (%)	4 (7.3%)	1 (2.1%)	3 (38%)	0.008
Casirivimab-imdevimab, n (%)	12 (22%)	12 (26%)	0 (0)	0.178
Bamlanivimab, n (%)	1 (1.8%)	1 (2.1%)	0 (0)	> 0.999
Tocilizumab, n (%)	4 (7.3%)	1 (2.1%)	3 (38%)	0.008
Favipiravir, n (%)	2 (3.6%)	1 (2.1%)	1 (13%)	0.272

CRRT/HD – continuous renal replacement therapy / hemodialysis
[1]Wilcoxon rank sum test; Fisher's exact test

this group of patients, there were no fatal outcomes; tocilizumab was used with four (7.3%) patients treated at the hospital (in which three of the cases ended up fatal). Antiviral (favipiravir) was used with two (3.6%) patients, where in one case, there was a fatal outcome (Figure 3).

The average value of serum creatinine before the patients had been infected with COVID-19 was 144 μmol/l, min: 90 μmol/l, max: 300 μmol/l. Three hospitalized patients (5.5% of those infected) experienced significant deterioration of transplanted kidney function, and therefore, they received renal function replacement therapy. All three of the patients developed multi-organ dysfunction syndrome and had passed away. The rest of the patients did not develop a significant decrease in graft function during the course of the illness, nor did they develop a month after the negative COVID-19 PCR test. The average serum creatinine after overcoming COVID-19 infection (n = 47) was 138.7 μmol/l (min: 88 μmol/l, max: 330 μmol/l) (Figure 4).

All of the patients were put back on immunosuppressive therapy after their inflammatory parameters and number of lymphocytes stabilized.

Out of all the ill (n = 55), fatal outcome occurred in eight (14.5%) patients, that is, when considering all of the patients being monitored after a KT (220), a fatal outcome caused by COVID-19 infection during the first two years of the pandemic was 3.6% (Table 2). All of the deceased patients were hospitalized at the time of their death. Regarding specific therapy one patient received favipiravir; three patients received tocilizumab; four patients did not receive tocilizumab/anti-SARS-CoV-2 mAb/antiviral; three patients received RRT. No deaths occurred among the patients who received casirivimab–imdevimab (n = 12) or bamlanivimab (n = 1). There is a statistically significant difference in survival between patients who received anti-SARS-CoV-2 mAb and patients who received tocilizumab ($p < 0.05$). The average age of patients who had a fatal outcome is 62.5 years old ($p < 0.05$), 75% of whom were male patients. Every patient with a fatal outcome received a cadaveric renal transplant.

The cause of death was severe acute respiratory syndrome in four patients (50%), multiorgan dysfunction syndrome in two patients (25%). All of the patients with

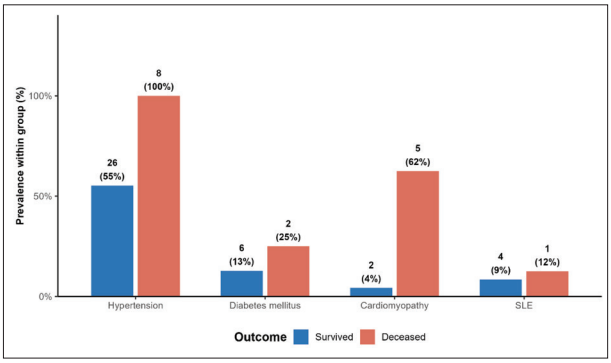


Figure 5. Comorbidities stratified by clinical outcome

fatal outcomes had serious comorbidities (congestive heart failure, diabetes mellitus, liver insufficiency, systemic lupus erythematosus). In two of the fatal outcomes, besides the comorbidity, the factor of late presentation to the hospital (more than seven days after disease beginning) without timely exclusion of antimetabolites and inclusion of the right therapy also took effect. Therefore, patients were hospitalized with well-developed massive pneumonia and signs of severe global respiratory insufficiency (Figure 5).

Statistically significant difference for death in patients with cardiomyopathy, hypertension, and late referral was confirmed compared to patients without these characteristics, $p < 0.05$ (Table 2).

DISCUSSION

In our study, we monitored 220 KTRs with a functioning graft between March 1, 2020, when the first patient in Serbia was diagnosed with COVID-19, and March 1, 2022. Out of all monitored KTRs, 55 (25%) were infected with COVID-19. The average age of those infected was 48.38 years old, 47% of patients received a deceased donor graft, 67% were male, and the average time period between the transplant and the infection of COVID-19 was 8.13 years. It was similar to a nationwide observational study of Swedish authors [2].

In our study, all of the patients were on triple immunosuppressive therapy, while 61% of patients in the group of Swedish authors were treated with three immunosuppressants as maintenance therapy [2]. Like most transplant centers, we modified immunosuppression in KTRs with COVID-19 infection; in 76% of the patients, the antimetabolite was excluded immediately upon COVID-19 diagnosis, the dose of corticosteroids was increased, while the calcineurin inhibitor dose was decreased by 20–25% [4].

The number of hospitalized patients in the Swedish study was 6.7% of all that have been monitored, which was less when compared to the results of our study: 18 patients (8.2%) (5.5% of patients in the first year, 2.7% of patients in the second year). In comparison to the group monitored by Swedish authors, the difference could be explained by better application of methods to reduce viral transmission, and thus the occurrence of infection. In our study, the most common group of infected patients was within a period of 1–5 years after transplantation (40%), which could be explained by the fact that the highest dosage of immunosuppressants was being used [2].

Swedish authors did not confirm a significant effect of comorbidities as independent risk factors, besides heart failure, but they confirmed a significant effect of diabetes mellitus as a primary diagnosis, as well as the effect of severe arterial hypertension. In our study, primarily congestive heart insufficiency, diabetes mellitus, systemic lupus erythematosus and liver insufficiency had a significant effect of comorbidities on the unfortunate outcome of the disease [2].

In our study, as well as in other studies, we found that bamlanivimab or casirivimab-imdevimab combined with immunosuppression reduction for outpatient treatment of mild to moderate COVID-19 infection in KTRs was safe and associated with good outcomes. Only two of the 13 patients treated with mAbs required hospitalization for COVID-19 because they developed pneumonia. Similar data was obtained from a study by Wang et al. [4], whose results showed a positive effect of mAbs use with the need for hospitalization in only four out of 27 patients treated with mAbs, without fatal outcome [5, 6, 7, 16].

In a systematic review and meta-analysis by Gatti et al. [21], the clinical outcome of COVID-19 infection in SOT recipients compared to generalized population was tested. However, in some studies, SOT recipients affected by COVID-19 showed a higher risk of mortality compared with the general population, especially with the presence of comorbidities (i.e., hypertension, diabetes mellitus, cardiovascular disease, chronic obstructive pulmonary disease, and chronic kidney disease) combined with old age. In our study, all of the patients who had a fatal outcome had some of the severe comorbidities (hypertension, diabetes mellitus, cardiovascular disease, congestive heart failure, systemic lupus erythematosus) [21].

Danziger-Isakov et al. [14] found that the mortality ranged from 9 to 46%, with most in the 18–30% range depending on the cohort and circumstance, when analyzing the impact of COVID-19 infection in SOT recipients. They also stated that many studies addressed risk factors for mortality with age, underlying cardiovascular or lung disease [14]. In our study, the mortality rate was 3.6% of all monitored KTRs, but all monitored were only KTRs.

In a French study, which included the first two months of the pandemic, it stated a high percentage of patients who developed AKI (45.8%), while using RRT in 13.2% of the cases [22, 23]. In our study, AKI developed in 5.5% of those ill, and all of them were on RRT. As in our study, factors combined with mortality were age (over 60 years old), male gender, arterial hypertension, cardiovascular diseases, and diabetes mellitus. However, in the said study, it had not been proven that KTRs are an independent factor combined with mortality [22, 23].

COVID-19 associated AKI is common, and has been associated with a higher mortality rate and was an independent risk factor for all-cause in-hospital death in COVID-19 patients. Mortality rate of 14.5% (of all COVID-19 infected

KTRs) in our study was similar to the one in Tavares et al. [24] (20%). Age and comorbidities were known risk factors associated with COVID-19 severity and higher mortality in the general population, as well as among SOT recipients. SOT recipients were predominantly 50 or older and had several comorbidities, including diabetes, hypertension, cardiovascular disease, chronic kidney disease, and obesity, which were associated with worse outcomes after COVID-19. Comorbidities rather than immunosuppression intensity were the main risk factors for impaired outcome in a large cohort [6, 7, 22, 25].

CONCLUSION

Firstly, a fortunate course of the disease was achieved by timely arrival at the institution of tertiary care that controls kidney transplant patients, with a modification of immunosuppressive therapy, and applying all therapeutic procedures in connection with the COVID-19 infection.

Secondly, therapeutic usage of bamlanivimab or casirivimab was reliable, with a significant positive effect on achieving mild and shorter courses of the disease, which allowed returning to the usual dosage of immunosuppressive therapy in a shorter amount of time.

The main risk factor for an unfavorable outcome of the COVID-19 infection in patients with a kidney transplant was presence of severe comorbidities and late presentation at the transplant center without timely modification of the immunosuppressive therapy and application of adequate therapeutic protocols.

Finally, the results obtained offer a critical review for improving the management of KTRs for any future pandemic or infectious disease on a larger scale.

Ethics: Before the start of the study, approval was granted by the Ethics Committee of the University Clinical Center of Vojvodina, Novi Sad, Serbia (Number: 00-50/2).

Conflict of interest: None declared.

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Исход инфекције ковидом 19 код болесника са трансплантираним бубрегом у прве две године пандемије – наша искуства са осврtом на примену тоцилизумаба и моноклонских *anti-SARS-CoV-2* антитела

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САЖЕТАК

Увод/Циљ Инфекција ковидом 19 код болесника са трансплантираним бубрегом била је повезана са већим ризиком од компликација и морталитета. Циљ рада био је да се испита учесталост и исход инфекције ковидом 19 код болесника контролисаних у нашем центру, као и ефекат тоцилизумаба и *anti-SARS-CoV*-2 моноклонских антитела (мАТ).

Методе Од укупно 220 болесника са трансплантираним бубрегом, испитали смо 55 болесника који су оболели од ковида 19 у прве две године пандемије.

Резултати Од укупног броја контролисаних болесника, у прве две године пандемије 55 (25%) болесника оболело је од ковида 19. Просечна старост оболелих била је 48,38 година. Имуносупресивна терапија није мењана код 24% болесника. Због развоја пнеумоније 33% болесника било је хоспитализовано. Од укупног броја оболелих, тоцилизумаб је примењен код 7% болесника, од којих су три преминула. *Anti-SARS-CoV*-2 мАТ примењена су код 24% болесника, при чему није било смртних исхода. Антивирусни лек (фавипиравир) примењен је код 4% болесника, а у једном случају наступио је смртни исход. Континуирана терапија замене бубрежне функције/хемодијализа примењена је код 5,5% болесника. Од укупног броја оболелих од ковида 19, смртни исход наступио је код 14,5%, а узрок смрти био је тежак акутни респираторни синдром (50%), срчани застој (25%) или мултиорганска слабост (25%).

Закључак Примена мАТ била је поуздана, са значајним позитивним ефектом на постизање блажег клиничког тока и краћег трајања болести. Главни фактори ризика за неповољне исходе инфекције ковидом 19 код прималаца трансплантираног бубрега били су озбиљни коморбидитети и касно јављање у центар за трансплантацију у узнапредовалој фази болести.

Кључне речи: трансплантација бубрега; инфекција ковидом 19; *anti-SARS-CoV*-2 антитела

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Inflammatory composite indicators and clinical features for prognostic factor analysis and malignancy prediction model construction in patients with glioma

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SUMMARY

Introduction/Objective The objective was to evaluate inflammatory composite indicators and clinical features as prognostic factors and develop a predictive model for glioma malignancy grade.

Methods A retrospective analysis of 107 patients with glioma who underwent surgical resection was conducted. Clinical data, including age, sex, body mass index, Karnofsky Performance Status, tumor characteristics and complete blood count parameters, were collected preoperatively and postoperatively. Cox proportional hazards regression was performed to identify independent prognostic factors for progression-free survival (PFS). The receiver operating characteristic curve analysis was used to evaluate the diagnostic performance of inflammatory markers in predicting high-grade glioma.

Results The cohort included 107 patients (mean age: 54.3 ± 13.5 years, 53.3% men). High-grade gliomas (World Health Organization grade IV) accounted for 55.1% of cases. Perioperative neutrophil counts increased significantly, whereas lymphocyte counts remained stable. In multivariate Cox analysis, younger age (hazard ratio 0.93, 95% confidence interval: 0.89–0.98, p = 0.009) was independently associated with improved PFS. The receiver operating characteristic analysis demonstrated that age (area under the curve [AUC] = 0.632, p = 0.019) and preoperative neutrophil-to-lymphocyte ratio (NLR) (AUC = 0.606, p = 0.058) showed potential in predicting high-grade glioma. Spearman correlation analysis revealed a significant negative correlation between Karnofsky Performance Status and NLR.

Conclusions Age represents an independent prognostic factor for PFS in patients with glioma. Preoperative inflammatory markers, particularly NLR, combined with clinical parameters such as age, show promise in predicting glioma malignancy grade.

Keywords: glioma; neutrophils; prognosis; inflammation

INTRODUCTION

Gliomas are the most common primary malignant brain tumors in adults, accounting for approximately 75% of all malignant central nervous system neoplasms [1]. Glioblastoma (GBM) (World Health Organization [WHO] grade IV) is the most aggressive subtype, with median overall survival (OS) of only 12–15 months despite surgery, radiotherapy, and temozolomide [2]. Reliable prognostic biomarkers are urgently needed.

Traditional prognostic factors include age, Karnofsky Performance Status (KPS), extent of resection (EOR) and molecular markers (e.g. isocitrate dehydrogenase [IDH] mutation, O6-methylguanine-DNA methyltransferase [MGMT] promoter methylation) [3, 4]. However, they often require complex testing or subjective assessment.

Inflammation is a cancer hallmark [5]. In gliomas, the tumor microenvironment involves complex interactions between tumor cells and inflammatory infiltrates, including neutrophils and lymphocytes [6]. The neutrophil-to-lymphocyte ratio (NLR), calculated as absolute neutrophil count divided by absolute lymphocyte count, reflects the balance between protumor inflammation and antitumor immunity.

Multiple studies demonstrate NLR's prognostic value in glioma. Chung et al. [7] reported that elevated NLR (≥ 5) following the standard Stupp protocol was associated with worse OS in GBM, suggesting that dynamic NLR changes may track recurrence. A meta-analysis by Yang et al. [8] confirmed that elevated NLR predicted worse OS in GBM (hazard ratio [HR] = 1.63, 95% confidence interval [CI]: 1.23–2.15, p = 0.0007). Higher NLR also correlates with increased tumor grade [7].

Perioperative stress and treatments alter peripheral blood counts. Han et al. [9] found that pretreatment NLR was associated with neutrophil and T-cell infiltration and predicted clinical outcomes in GBM. The EOR remains a key modifiable factor: gross total resection (GTR) improves survival compared with subtotal resection (STR) or biopsy [10, 11].

Functional status, measured by KPS (0–100), is another crucial determinant. Postoperative KPS has superior predictive value over preoperative KPS [12]. Recent research has emphasized

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the importance of maintaining KPS ≥ 50%, which allows patients to live at home and maintain personal care activities, as a key quality-of-life endpoint [13].

Despite NLR's established value, significant gaps remain: perioperative inflammatory dynamics are poorly characterized, the relationship between systemic inflammation and KPS has not been systematically explored and the integrated utility of inflammatory markers for preoperative grade prediction is uncertain. Addressing these gaps could improve preoperative risk stratification and treatment planning.

This study aimed to evaluate the prognostic significance of inflammatory composite indicators and clinical features for progression-free survival (PFS); to analyze perioperative changes in inflammatory markers; to develop a predictive model distinguishing high- from low-grade gliomas using pre-treatment parameters; to explore correlations between inflammatory markers and clinical features to better understand systemic inflammation in glioma.

METHODS

Study design and patient population

This retrospective study included consecutive patients who underwent surgical resection for histologically confirmed glioma at a tertiary center (from January 2017 until December 2025). The institutional ethics committee approved the protocol; informed consent was waived.

The inclusion criteria included age ≥ 18 years, histopathologically confirmed glioma (WHO classification), complete blood count data within seven days preoperatively and postoperatively and complete clinical and follow-up data. The exclusion criteria included prior brain surgery/radiotherapy, active infection/inflammatory disease at surgery, the use of immunosuppressants or corticosteroids (> 8 mg/day dexamethasone equivalent) for > 7 days preoperatively, hematologic disorders and incomplete data.

Data collection and variable definition

Clinical data from electronic medical records included age, sex, body mass index (BMI) (kg/m²), smoking status and preoperative KPS (assessed within 24 hours; scale 0–100, ≥ 80 = normal activity with minor symptoms, < 70 = requires assistance). Additional variables included neurological symptoms, symptom duration and antiepileptic use.

Tumor characteristics on preoperative magnetic resonance imaging (MRI) included maximum diameter (contrast-enhanced T1 or T2-FLAIR), location (lobe, hemisphere), eloquent area involvement (motor/sensory/language/visual cortex, deep grey matter), multifocality and contrast enhancement. Histopathology, as per WHO specification, was low-grade (II), intermediate (III) and high-grade (IV).

Surgical variables included EOR – GTR (≥ 95% removal on postoperative MRI within 48 hours) or STR (75–94% removal) – determined via volumetric analysis (three-dimensional reconstruction).

Laboratory parameters (within seven days pre/postoperatively) included absolute neutrophil, lymphocyte and monocyte counts ($\times 10^9$/L); platelet count ($\times 10^9$/L); NLR; and platelet/lymphocyte ratio (PLR).

Treatment and follow-up

Treatment included maximal safe resection guided by neuronavigation and neurophysiological monitoring. Postoperative treatment was performed by a multidisciplinary board, including adjuvant radiotherapy (60 Gy/30 fractions for high grade, 50.4–54 Gy for intermediate grade) and chemotherapy (temozolomide or other).

The follow-up was every three months for two years, then every six months, and included clinical examination, KPS and MRI. Disease progression was defined in accordance with Response Assessment in Neuro-Oncology criteria. Progression-free survival was defined as time from surgery to progression or death; OS was defined as time from surgery to death or last follow-up.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR) based on normality (Shapiro–Wilk test). Categorical variables were expressed as frequencies/percentages. A paired t-test or Wilcoxon's signed-rank test was used to compare preoperative and postoperative blood parameters. An independent t-test or the Mann–Whitney U test was used for two-group comparisons; a χ^2 test or Fisher's exact test was used for categorical variables.

Univariate Cox proportional hazards regression identified potential prognostic factors for PFS. Variables with $p < 0.20$ in univariate analysis, plus clinically recognized prognostic factors, entered the multivariate Cox (backward elimination) regression analysis. Hazard ratios with 95% CIs were calculated (HR > 1: increased risk; HR < 1: protective effect). The proportional hazards assumption was verified using Schoenfeld residuals.

Kaplan–Meier curves visualized PFS; log-rank tests were used to compare survival distributions. Optimal cut-offs for continuous variables were determined via maximally selected rank statistics or receiver operating characteristic (ROC) curve analysis. The ROC analysis evaluated the diagnostic performance of inflammatory markers and clinical variables for predicting high-grade gliomas (WHO grade IV) versus low- or intermediate-grade gliomas. Area under the curve (AUC) values with 95% CIs were calculated; the optimal cut-off was determined using Youden's index (sensitivity+specificity−1). Sensitivity, specificity, positive predictive value and negative predictive value were calculated for each significant predictor.

Spearman's rank correlation coefficients were used to assess relationships between inflammatory markers and clinical features. Correlation strength was weak ($r < 0.3$), moderate ($0.3 \leq r < 0.7$) or strong ($r \geq 0.7$). Correlation matrices with heatmaps were generated.

Table 1. Baseline characteristics of the study population (n = 107)

Variable	Value
Demographics	
Age (years), mean ± SD	54.3 ± 13.5
Age (years), median (range)	55 (21–82)
Sex, male, n (%)	57 (53.3)
Sex, female, n (%)	50 (46.7)
Body mass index (kg/m²), mean ± SD	24.7 ± 3.2
Clinical features	
Preoperative KPS, mean ± SD	79.2 ± 29.7
Preoperative KPS, median (interquartile range)	95 (80–95)
KPS ≥ 80, n (%)	72 (67.3)
KPS < 80, n (%)	35 (32.7)
Tumor characteristics	
Tumor size (cm), mean ± SD	5.45 ± 2.08
Tumor location	
- Frontal lobe, n (%)	41 (38.3)
- Temporal lobe, n (%)	35 (32.7)
- Parietal lobe, n (%)	18 (16.8)
- Other locations, n (%)	13 (12.2)
Eloquent location, n (%)	48 (44.9)
Pathology	
Low grade (II), n (%)	26 (24.3)
Mid grade (III), n (%)	22 (20.6)
High grade (IV), n (%)	59 (55.1)
Surgical Details	
Extent of resection	
- gross total resection (≥ 95%), n (%)	69 (64.5)
- subtotal resection (75–94%), n (%)	38 (35.5)
Preoperative laboratory values	
Neutrophils (× 10⁹/L), mean ± SD	4.81 ± 2.54
Lymphocytes (× 10⁹/L), mean ± SD	1.66 ± 0.68
Platelets (× 10⁹/L), mean ± SD	223 ± 71.4
Neutrophil-to-lymphocyte ratio, mean ± SD	4.21 ± 8.05
Neutrophil-to-lymphocyte ratio, median (interquartile range)	2.85 (1.98–4.52)
Platelet-to-lymphocyte ratio, mean ± SD	162.11 ± 102.02
Adjuvant therapy	
Radiotherapy, n (%)	28 (26.2)
Chemotherapy, n (%)	26 (24.3)

SD – standard deviation; KPS – Karnofsky performance status

All tests were two tailed, with $p < 0.05$ indicating statistical significance. Analyses were conducted using R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria) with the survival, survminer, pROC and corrplot packages.

RESULTS

Baseline characteristics

A total of 107 patients were included in the final analysis (Table 1). The mean age at diagnosis was 54.3 ± 13.5 years, with 57 male patients (53.3%) and 50 female patients (46.7%). The mean BMI was 24.7 ± 3.2 kg/m²; the mean preoperative KPS was 79.2 ± 29.7 (median: 95). Tumor pathology results were as follows: high-grade (WHO IV) 55.1% (n = 59), intermediate (III) 20.6% (n = 22) and low-grade (II) 24.3% (n = 26). The mean tumor diameter was 5.45 ± 2.08 cm. Frontal lobe tumors were the most common type (38.3%), followed by temporal tumors (32.7%). The eloquent location percentage was 44.9%; GTR was identified in 64.5% of cases and STR in 35.5%.

Preoperative neutrophil count was 4.81 ± 2.54 × 10⁹/L, lymphocyte count was 1.66 ± 0.68 × 10⁹/L and platelet count was 223 ± 71.4 × 10⁹/L. The mean NLR was 4.21 ± 8.05 (median: 2.85, IQR: 1.98–4.52), and the mean PLR was 162.11 ± 102.02. Adjuvant radiotherapy was performed in 28 (26.2%) cases, and chemotherapy in 26 (24.3%), typically for high-grade or incompletely resected tumors.

Perioperative changes in inflammatory markers

Paired analysis (Table 2, Figure 1) showed a significant postoperative neutrophil increase from 4.81 ± 2.54 to 6.57 ± 3.21 × 10⁹/L (mean difference: +1.76, $p < 0.001$). Lymphocytes decreased non-significantly from 1.66 ± 0.68 to 1.6 ± 0.71 × 10⁹/L ($p = 0.391$). Platelets increased from 223 ± 71.4 to 229 ± 88.2 × 10⁹/L ($p = 0.418$), and NLR

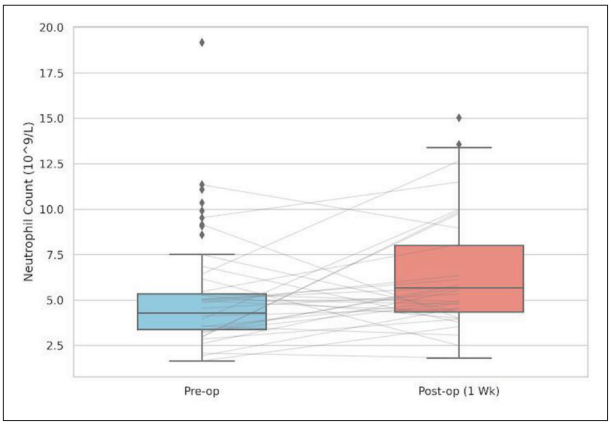


Figure 1. Paired comparison of perioperative neutrophil counts

Table 2. Comparison of preoperative and postoperative blood indices

Indicator	Pre-op mean ± SD	Post-op mean ± SD	Mean difference	95% CI	p	Interpretation
Neutrophils (× 10⁹/L)	4.81 ± 2.54	6.57 ± 3.21	+1.76	1.02–2.50	< 0.001*	Significant increase
Lymphocytes (× 10⁹/L)	1.66 ± 0.68	1.6 ± 0.71	-0.06	-0.18–0.06	0.391	No significant change
Platelets (× 10⁹/L)	223 ± 71.4	229 ± 88.2	+6	-7.2–19.2	0.418	No significant change
Neutrophil-to-lymphocyte ratio	4.21 ± 8.05	4.77 ± 3.94	+0.57	-0.91–2.05	0.466	No significant change
Platelet-to-lymphocyte ratio	162.11 ± 102.02	169.34 ± 115.28	+7.23	-12.45–26.91	0.512	No significant change

SD – standard deviation; CI – confidence interval; *statistically significant at $p < 0.05$

increased from 4.21 to 4.77 (p = 0.466). These findings reflect surgical stress and highlight timing considerations for inflammatory marker assessment.

Univariate analysis of prognostic factors for progression-free survival

Univariate Cox regression (Table 3) identified trends towards better PFS with younger age (HR = 0.97 per year, 95% CI: 0.93–1.01, p = 0.151) and GTR versus STR (HR = 0.49, p = 0.302), and worse prognosis with high-grade pathology (HR = 2.34, p = 0.166). However, none reached statistical significance. Male sex (HR = 0.69, p = 0.508), BMI (HR = 0.97, p = 0.763), preoperative KPS (HR = 1, p = 0.589), tumor size (HR = 1.07, p = 0.637), neutrophil count (HR = 1.12, p = 0.188), NLR (HR = 1, p = 0.924) and PLR (HR = 1, p = 0.782) showed no significant associations. Adjuvant radiotherapy (HR = 6.42, 95% CI: 1.76–23.40, p = 0.005) and chemotherapy (HR = 7.69, 95% CI: 2.09–28.33, p = 0.002) were paradoxically associated with worse PFS, reflecting selection bias (these therapies were preferentially given to patients at higher risk).

Table 3. Univariate Cox regression analysis for progression-free survival

Variable	Hazard ratio	95% CI	p
Age (per year)	0.97	0.93–1.01	0.151
Sex (male *vs.* female)	0.69	0.23–2.05	0.508
Body mass index (per kg/m^2)	0.97	0.79–1.18	0.763
Preoperative Karnofsky performance status (per point)	1.00	0.98–1.01	0.589
Tumor size (per cm)	1.07	0.80–1.45	0.637
Resection (GTR *vs.* STR)	0.49	0.13–1.91	0.302
Pathology (high *vs.* low/mid)	2.34	0.70–7.80	0.166
Eloquent location (yes *vs.* no)	1.12	0.38–3.28	0.838
Preoperative neutrophils	1.12	0.95–1.32	0.188
Preoperative lymphocytes	0.88	0.45–1.73	0.715
Preoperative NLR	1.00	0.94–1.07	0.924
Preoperative PLR	1.00	0.99–1.01	0.782
Radiotherapy (yes *vs.* no)	6.42	1.76–23.40	0.005*
Chemotherapy (yes *vs.* no)	7.69	2.09–28.33	0.002*

CI – confidence interval; GTR – gross total resection; STR – subtotal resection; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; *statistically significant at p < 0.05

Table 4. Multivariate Cox proportional hazards model for progression-free survival

Variable	Hazard ratio	95% CI	p
Age (per year)	0.93	0.89–0.98	0.009*
Pathology (high *vs.* low/mid)	4.67	0.48–45.41	0.185
Resection (gross total resection *vs.* subtotal resection)	0.97	0.06–15.12	0.980
Preoperative Karnofsky performance status (per point)	0.99	0.97–1.01	0.252
Preoperative neutrophil-to-lymphocyte ratio	0.99	0.92–1.08	0.902

*statistically significant at p < 0.05

Multivariate Cox regression analysis

Variables with p < 0.20 (age, pathology grade, resection extent, preoperative KPS and neutrophil count / NLR) were entered into multivariate Cox analysis with backward elimination. The final model retained age as the only independent prognostic factor (Table 4). Younger age independently predicted better PFS (HR = 0.93 per year, 95% CI: 0.89–0.98, p = 0.009); each additional year increased the progression hazard by approximately 7%. Pathology grade (p = 0.185), EOR (p = 0.980), KPS (p = 0.252) and NLR (p = 0.902) were not independently significant.

Survival analysis by clinical and pathological factors

Kaplan–Meier curves (Figures 2 and 3) demonstrated clear PFS stratification by pathology grade (log-rank p < 0.001).

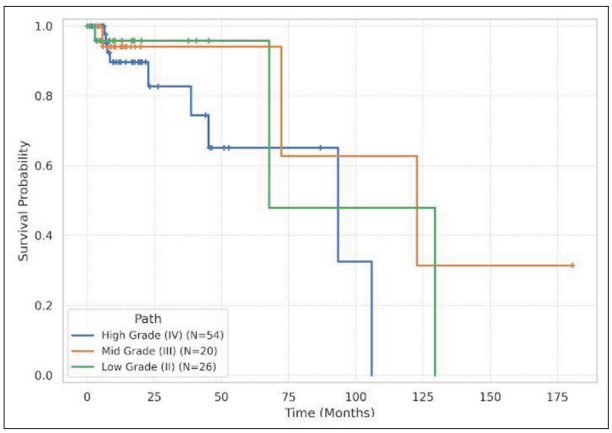


Figure 2. Kaplan-Meier survival analysis by pathology grade; the survival curves demonstrate significant differences in progression-free survival across glioma grades; high-grade [World Health Organization (WHO) grade IV] gliomas (blue line) show the most rapid decline in survival probability, with median progression-free survival of approximately eight months; mid-grade (WHO grade III, brown line) and low-grade (WHO grade II, green line) gliomas demonstrate considerably better survival outcomes; the "+" symbols indicate censored observations; log-rank test p < 0.001

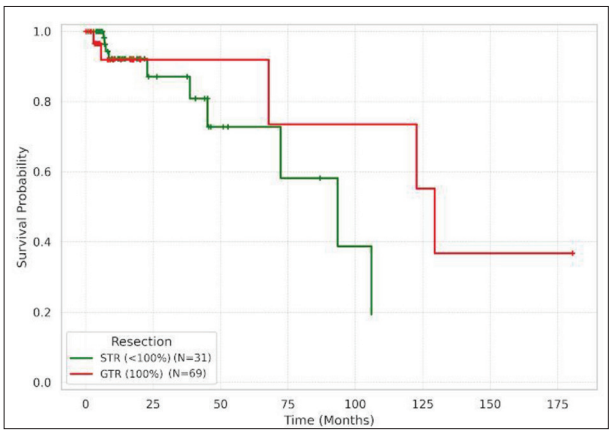


Figure 3. Impact of resection extent on progression-free survival; Kaplan–Meier curves comparing patients who underwent gross total resection [(GTR), red line] *vs.* subtotal resection [(STR), green line]; GTR patients demonstrate consistently higher survival probabilities throughout follow-up, with approximately 70% progression-free at 12 months compared to 55% for STR; log-rank test p = 0.089

Table 5. Diagnostic performance of inflammatory markers and clinical features for predicting high-grade glioma

Variable	AUC	95% CI	p	Cutoff	Sensitivity	Specificity
Age (years)	0.632	0.527–0.737	0.019*	> 58	56.5%	77.3%
Preoperative NLR	0.606	0.499–0.713	0.058	> 1.76	85.5%	34.1%
Preoperative PLR	0.542	0.433–0.651	0.456	> 124.55	68.1%	47.7%
Tumor size (cm)	0.526	0.417–0.635	0.641	> 4	82.6%	22.7%
Preoperative KPS	0.476	0.366–0.586	0.667	≤ 90	62.7%	47.9%

AUC – area under the curve; CI – confidence interval;
NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio;
KPS – Karnofsky performance status;
*statistically significant at p < 0.05

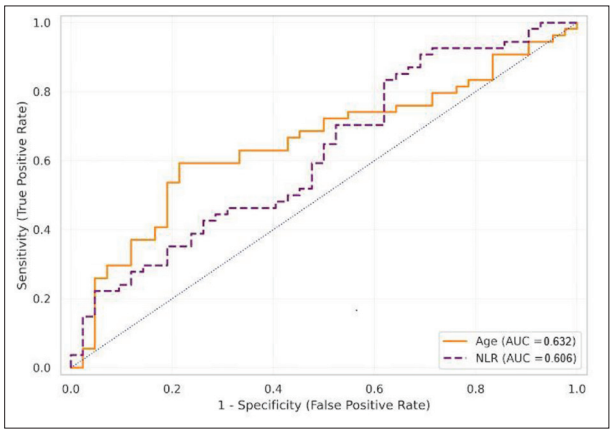


Figure 4. Diagnostic ROC curve analysis for predicting high-grade glioma; the stepped ROC curves display the diagnostic performance of age (orange solid line, AUC = 0.632) and preoperative neutrophil-to-lymphocyte ratio (purple dashed line, AUC = 0.606) in distinguishing high-grade (World Health Organization grade IV) from lower-grade gliomas; the characteristic stepped appearance reflects the finite sample size typical of real-world clinical datasets; the diagonal reference line represents random chance (AUC = 0.5); both markers showed modest but statistically significant or near-significant predictive value

High-grade (WHO IV) gliomas had a median PFS of approximately eight months, whereas lower-grade tumors showed substantially longer survival. Regarding EOR, patients with GTR had higher survival probabilities at all time points than those with STR (12-month PFS: approx. 70% *vs.* 55%), but the log-rank test was not significant (p = 0.089).

Table 6. Spearman correlation matrix of clinical features

Variables	Correlation coefficient (r)	p	Interpretation
KPS *vs.* NLR	-0.453	< 0.001*	Moderate negative correlation
Age *vs.* KPS	-0.387	< 0.001*	Moderate negative correlation
NLR *vs.* PLR	+0.632	< 0.001*	Strong positive correlation
Tumor size *vs.* NLR	-0.110	0.259	No significant correlation
Tumor size *vs.* KPS	+0.088	0.368	No significant correlation
Age *vs.* NLR	+0.156	0.109	Weak positive correlation

NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio;
KPS – Karnofsky performance status;
*statistically significant at p < 0.05

Table 7. Impact of adjuvant therapy on progression-free survival (subgroup analysis)

Treatment	N yes/no	Hazard ratio	95% CI	p	Interpretation
Radiotherapy	28/79	6.42	1.76–23.40	0.005*	Selection bias effect
Chemotherapy	26/81	7.69	2.09–28.33	0.002*	Selection bias effect

CI – confidence interval;
*statistically significant (reflects selection bias rather than treatment harm)

Diagnostic performance of inflammatory markers for predicting high-grade glioma

The ROC analysis (Table 5, Figure 4) evaluated preoperative markers for predicting high-grade (WHO IV) glioma. Age showed moderate discriminative ability (AUC = 0.632, 95% CI: 0.527–0.737, p = 0.019); an optimal cut-off > 58 years yielded a sensitivity of 56.5% and a specificity of 77.3%. Preoperative NLR showed borderline significance (AUC = 0.606, 95% CI: 0.499–0.713, p = 0.058); a cut-off > 1.76 yielded high sensitivity (85.5%) but low specificity (34.1%); PLR (AUC = 0.542, p = 0.456), tumor size (AUC = 0.526, p = 0.641) and preoperative KPS (AUC = 0.476, p = 0.667) showed no significant discriminative ability.

Correlation analysis of clinical features

Spearman correlation analysis (Table 6) revealed a significant negative correlation between preoperative KPS and NLR (r = -0.453, p < 0.001). Age also correlated negatively with KPS (r = -0.387, p < 0.001); NLR and PLR showed a strong positive correlation (r = 0.632, p < 0.001). Tumor size correlated with neither NLR (r = -0.110, p = 0.259) nor KPS (r = 0.088, p = 0.368).

Impact of adjuvant therapy

As shown in Table 7, adjuvant radiotherapy (HR = 6.42, 95% CI: 1.76–23.40, p = 0.005) and chemotherapy (HR = 7.69, 95% CI: 2.09–28.33, p = 0.002) were paradoxically associated with worse PFS, reflecting confounding by indication. Among radiotherapy recipients, 89.3% had high-grade gliomas compared with 42% of non-recipients (p < 0.001). Chemotherapy recipients had larger tumors (6.2 ± 2.3 *vs.* 5.1 ± 1.9 cm, p = 0.012) and lower GTR rates (46.2% *vs.* 72.8%, p = 0.009).

DISCUSSION

The present study evaluated clinical, pathological, treatment-related and systemic inflammatory factors in relation to PFS and the preoperative identification of high-grade glioma. Age was the only variable retained as an independent factor in the multivariable Cox model (HR = 0.93, 95% CI: 0.89–0.98, p = 0.009), confirming the importance of age in prognostic assessment [14]. Pathological grade clearly stratified PFS in the Kaplan–Meier analysis, whereas GTR showed

a favorable but statistically nonsignificant survival trend. Preoperative NLR demonstrated limited, borderline discriminatory ability for high-grade glioma and was significantly associated with functional status. Taken together, these findings suggest that prognosis is not determined by a single marker but by the interaction of tumor aggressiveness, host functional reserve, systemic inflammation, surgical treatment and subsequent treatment selection.

Age, KPS and systemic inflammatory indices should therefore be interpreted as related rather than isolated prognostic domains. Age was moderately and negatively correlated with preoperative KPS (r = -0.387, $p < 0.001$), indicating that older patients tend to have poorer functional status. In parallel, NLR was moderately and negatively correlated with KPS (r = -0.453, $p < 0.001$), suggesting that a greater systemic inflammatory burden was associated with reduced functional capacity. However, the direct correlation between age and NLR was weak and not statistically significant (r = 0.156, $p = 0.109$). This pattern is compatible with, but does not prove, a clinical pathway in which age-related loss of physiological reserve and systemic inflammation jointly contribute to poorer performance status. Because these associations were cross-sectional, KPS cannot be considered a mediator between age, inflammation and PFS on the basis of the present data alone. Moreover, NLR and PLR were strongly correlated (r = 0.632, $p < 0.001$), which is biologically plausible because both indices reflect systemic inflammation and share lymphocyte count as their denominator. Their correlation also indicates that they should not automatically be treated as independent sources of prognostic information. By contrast, tumor size was not correlated with either NLR or KPS, suggesting that systemic inflammation and functional impairment were not simply proxies for radiological tumor size.

The perioperative findings further emphasize the importance of measurement timing. Neutrophil counts increased significantly after surgery, whereas lymphocyte and platelet counts did not change significantly. Although the mean NLR increased numerically from 4.21 to 4.77, this change was not statistically significant ($p = 0.466$). Therefore, the present results support a postoperative neutrophil response to surgical stress but do not establish a significant perioperative increase in NLR. Previous evidence suggests that a marked rise in NLR following tumor resection may be associated with poorer outcomes in GBM [15]. Prospective studies using standardized sampling intervals are needed to determine whether perioperative NLR trajectories provide information beyond baseline inflammatory status and established clinical factors.

In our ROC analysis, preoperative NLR showed moderate predictive value for high-grade glioma (AUC = 0.606), consistent with prior reports. A systematic review confirmed that elevated NLR is consistently associated with high-grade gliomas, although optimal cut-offs varied across studies [16]. Our identified cut-off of 1.76 is lower than many published thresholds, possibly due to differences in study populations, grade distributions or laboratory methods. At this cut-off, NLR demonstrated high sensitivity (85.5%) but low specificity (34.1%), suggesting it may be more useful as a negative predictive marker than a definitive diagnostic tool.

NLR was negatively correlated with preoperative KPS (r = -0.453, $p < 0.001$), suggesting that systemic inflammation contributes to functional decline. Prior research has also reported inverse correlations between KPS and inflammatory markers and has shown that baseline functional status predicts postoperative improvement [17]. Understanding this relationship may enable clinicians to identify patients at risk for poor functional outcomes and potentially implement prehabilitation strategies, although such interventions remain investigational.

EOR showed a favorable but nonsignificant survival trend ($p = 0.089$), contrasting with literature supporting maximal safe resection [18]. Several explanations may account for this discrepancy. First, our relatively modest sample size may have limited statistical power to detect significant differences. Second, the dichotomization of EOR into GTR compared with STR may oversimplify a more nuanced relationship. Third, the prognostic benefit of extensive resection may be most pronounced in specific molecular subtypes, and our study did not stratify by IDH mutation status or other molecular markers due to incomplete data availability.

Despite promising univariate trends, preoperative NLR was not independently prognostic in our multivariate Cox model, diverging from meta-analyses showing that elevated NLR predicts worse OS in GBM [8]. Possible explanations are that our heterogeneous cohort included all glioma grades, whereas supportive literature focuses on GBM; NLR's prognostic value may be grade dependent [19]; and post-treatment or dynamic NLR changes may be more informative than static preoperative values [20].

The paradoxical association of adjuvant radiotherapy/chemotherapy with worse PFS reflects confounding by indication, as these therapies were preferentially given to patients at higher risk. This well-documented phenomenon underscores the need for appropriate statistical adjustment and cautious interpretation. Future studies using propensity score matching may help address such selection bias.

Inflammatory markers could serve as surrogate biomarkers for treatment response and surveillance. Unlike imaging, peripheral blood markers offer objective, non-invasive and cost-effective monitoring [21]. Serial NLR measurements may detect early progression before radiological changes.

Mechanistically, neutrophils promote tumor progression via matrix metalloproteinases and vascular endothelial growth factor and suppress antitumor immunity [22]. Lymphocytes mediate antitumor responses, and lymphopenia reflects impaired immunity [23]. Thus, NLR represents a clinically measurable proxy for tumor-immune equilibrium.

Immunotherapies (e.g., immune checkpoint inhibitors, CAR T-cell therapy) have limited success [24]. Baseline inflammation may predict response, and elevated NLR might indicate poor benefit. Inflammatory markers could become companion biomarkers.

The clinical translation of inflammatory markers as prognostic tools requires careful consideration of cut-off values, measurement standardization and integration into decision-making frameworks. Unlike molecular markers, which typically have biologically defined thresholds, inflammatory markers are continuous variables subject to laboratory variation and influenced by numerous confounding factors, including infection, stress, medications and comorbidities [25]. Efforts to standardize collection protocols, define universal cut-offs through large prospective studies and develop validated algorithms will be essential for clinical implementation.

This study has several limitations. First, the retrospective single-center design may have introduced selection bias and limit generalizability. Second, the modest sample size lacked sufficient statistical power for subgroup analyses, and the heterogeneous follow-up durations further limited comprehensive survival assessment. Notably, molecular marker data (IDH mutation, MGMT promoter methylation) were unavailable, despite the 2021 WHO classification emphasizing their fundamental role in glioma diagnosis and prognostication [26]; IDH mutation and MGMT methylation strongly influence survival and treatment response [27]. Additionally, we did not evaluate post-treatment inflammatory marker dynamics or their association with disease recurrence, which warrant future investigation.

Despite these limitations, our study provides valuable insights into the interplay between systemic inflammation, clinical features and glioma prognosis. The demonstration of a significant correlation between NLR and functional status provides biological plausibility for the use of inflammatory markers as prognostic tools. The independent prognostic value of age reinforces its importance in contemporary glioma management. The modest but significant predictive value of age and NLR for high-grade pathology suggests potential utility in preoperative risk stratification and surgical planning.

CONCLUSION

In conclusion, the present findings support a multidimensional interpretation of prognosis in glioma. Age provided independent prognostic information in the multivariable model, pathological grade reflected tumor aggressiveness, EOR represented a potentially modifiable treatment factor and KPS reflected the patients' functional reserve. Furthermore, NLR and PLR characterized systemic inflammatory status but were closely related to each other and should not be regarded as independent predictors. The association between higher NLR and lower KPS suggests a clinically relevant connection between systemic inflammation and functional impairment, whereas the absence of correlations with tumor size indicates that these measures capture information not provided by tumor dimensions alone. Given the limited discrimination of individual markers, future models should integrate clinical, pathological, molecular, treatment-related and longitudinal inflammatory variables and should undergo external validation before being used for individual patient counselling or treatment selection.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Hebei YanDa Hospital. Due to the nature of retrospective study and anonymized patient information, informed consent is waived with the approval of Ethics Committee of Hebei YanDa Hospital.

Availability of data and materials

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Authors' contributions

Wang BH and He YC conceived of the study, and Wang BH and He YC participated in its design and data analysis and statistics and Wang BH and He YC helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of interest: None declared.

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Инфламаторни композитни индикатори и клиничке карактеристике за анализу прогностичких фактора и изградњу модела за предвиђање малигнитета код болесника са глиомом

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САЖЕТАК

Увод/Циљ Циљ рада био је процена инфламаторних композитних индикатора и клиничких карактеристика као прогностичких фактора и развој модела за предвиђање степена малигнитета глиома.

Методе Спроведена је ретроспективна анализа 107 болесника са глиомом који су подвргнути хируршкој ресекцији. Прикупљени су клинички подаци, укључујући старост, пол, индекс телесне масе, Карнофски индекс, карактеристике тумора и параметре комплетне крвне слике, преоперативно и постоперативно. Коксова регресија пропорционалних ризика примењена је за идентификацију независних прогностичких фактора преживљавања без прогресије. Анализа *ROC* криве коришћена је за процену дијагностичких перформанси инфламаторних маркера у предвиђању глиома високог степена малигнитета.

Резултати Кохорта је обухватила 107 болесника (средња старост 54,3 ± 13,5 година, 53,3% мушкараца). Глиоми високог степена малигнитета (IV степен према класификацији Светске здравствене организације) чинили су 55,1% случајева. Број неутрофила у периоперативном периоду значајно је порастао, док је број лимфоцита остао стабилан. У мултиваријантној Коксовој анализи, млађа старост (*HR* = 0,93, 95% *CI* 0,89–0,98, *p* = 0,009) била је независно повезана са бољим преживљавањем без прогресије болести; *ROC* анализа показала је да старост (*AUC* = 0,632, *p* = 0,019) и преоперативни однос неутрофила и лимфоцита (*AUC* = 0,606, *p* = 0,058) показују потенцијал у предвиђању глиома високог степена малигнитета. Спирманова корелациона анализа открила је значајну негативну корелацију између Карнофског индекса и односа неутрофила и лимфоцита.

Закључак Старост представља независни прогностички фактор преживљавања без прогресије болести код болесника са глиомом. Преоперативни инфламаторни маркери, посебно однос неутрофила и лимфоцита, у комбинацији са клиничким параметрима, као што је старост, показују потенцијал у предвиђању степена малигнитета глиома.

Кључне речи: глиом; неутрофили; прогноза; упала

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Can heart rate variability be utilized to predict compensatory sweating after endoscopic thoracic sympathicotomy in patients with primary focal hyperhidrosis?

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SUMMARY

Introduction/Objective Hyperhidrosis is a condition of pathologically excessive sweating that exceeds the body's physiological requirements for thermoregulation. Endoscopic thoracic sympathicotomy (ETS) is a commonly used treatment for primary focal hyperhidrosis (PFH); however, it may result in compensatory sweating (CS), which represents its most frequent postoperative complication. This study aimed to investigate whether heart rate variability (HRV) parameters could predict CS.

Methods A prospective single-center study was conducted in 100 patients with PFH who underwent ETS. Preoperatively, all patients underwent 24-hour Holter electrocardiographic monitoring to assess standard HRV parameters. ETS was performed by transection of the sympathetic chain at the level of the third (R3) and fourth (R4) ribs, including the accessory Kuntz fibers. One month after surgery, the presence and severity of CS were assessed.

Results CS was absent in 61% of patients ($p = 0.028$). Mild CS was the most common form ($p < 0.01$). No statistically significant differences were observed in the analyzed preoperative HRV parameters between patients with and without CS ($p > 0.05$), including the low-frequency (LF)/high-frequency (HF) ratios, which represent balanced autonomic activity (LF/HF < 2) and sympathetic predominance (LF/HF ≥ 2). Receiver operating characteristic curve analysis showed that age had significant predictive accuracy for CS (area under the curve 0.7333; 95% confidence interval 0.634–0.833; $p < 0.01$), with an optimal cut-off of 23.5 years (sensitivity 0.82, specificity 0.47).

Conclusion HRV parameters cannot reliably predict CS following bilateral ETS in patients with PFH. The study confirmed that the optimal age for surgery is below 23.5 years.

Keywords: video-assisted thoracic surgery; sweating; sympathectomy; autonomic nervous system; minimally invasive surgery

INTRODUCTION

Hyperhidrosis is defined as a condition of pathologically excessive sweating that exceeds the body's physiological needs for thermoregulation. Hyperhidrosis can be secondary, most often generalized, as a consequence of various diseases and conditions, or primary, focal or cryptogenic, with involvement of the palms, soles or axillae. The pathophysiology of primary focal hyperhidrosis (PFH) remains unclear. Excessive sweating in PFH is a direct consequence of increased secretion by sweat glands. Eccrine glands in individuals with PFH are completely normal in anatomy, number, and distribution [1]. It has been suggested that PFH results from complex hyperstimulation of the sympathetic nervous system (SNS) when exposed to emotional stress. Any emotional state or exposure to stress can be an activator of an episode of PFH, but symptoms can also occur without any emotional or thermal stimuli. After the action of an activating stimulus, sweating occurs instantly and is continuous. If conservative treatment fails, endoscopic thoracic sympathicotomy (ETS) is the method of choice [2, 3]. The most common complication of ETS is compensatory sweating (CS) [4]. CS affects regions of the body that have not been previously surgically altered. The mechanism of action is still unknown [5]. Some research also shows a high prevalence of depression and anxiety disorders in this group, along with specific dietary habits, including increased caffeine consumption and the recognition of spicy and fatty foods as sweating triggers [6, 7]. Expert consensus stated that, to prevent CS, patients undergoing surgery should preferably be under

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25 years of age and have a body mass index under 28 [3, 8]. Neuronal cell bodies responsible for the innervation of the sweat glands of the trunk and upper extremities are located at the level of the second to eighth thoracic segments of the spinal cord [9]. With the current evidence level, it is generally accepted that most cardiac sympathetic fibers also originate from the spinal cord at the level of the first to fifth thoracic segments [10, 11].

Heart rate variability (HRV) is a non-invasive, easily reproducible method of measuring the influence of the autonomic nervous system on cardiac function. The data were obtained through 24-hour Holter monitoring and subsequent computer processing. HRV indices provide numerical values of cardiac autonomic nervous system tone. The basic principle of HRV is the measurement of the change in the interval between two consecutive heartbeats – rhythm fluctuations [12]. Research worldwide indicates that patients with hyperhidrosis experience a significant decrease in quality of life. This reduction is reflected in lower scores on the mental component of the SF-36 questionnaire and decreased values on the Dermatology Life Quality Index [13, 14]. However, after treatment of primary hyperhidrosis, the quality of life of patients improves significantly, and this improvement is maintained for many years after the intervention [15, 16]. This is shown by global meta-analyses and observations by domestic authors [17].

The aim of this study was to confirm the potential existence of HRV parameters that would serve as predictors of CS.

METHODS

A prospective, monocentric, non-interventional study was conducted at the Institute of Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia, in the period from March 2022 to July 2024. After voluntarily signing the informed consent form, 100 subjects were included in the study. Patients were divided into two equal groups (50 patients each) according to the number of affected regions: palmar and plantar (PP) PFH, and palmar, plantar and axillary (PPA) PFH. Inclusion criteria were: first operation for PFH, Hyperhidrosis Disease Severity Scale grades three and four. Exclusion criteria were the following: psychiatric diseases, cardiac diseases and use of drugs, acute infections, use of alcohol, narcotics and tobacco, professional sports. Holter monitoring for 24 hours was undertaken preoperatively using EVO 4 (Spacelabs Healthcare Ltd, Hertford, UK) with Spacelabs Healthcare 2018 – Sentinel v11.0.0.9904 data processing software. All standard HRV parameters in the time and frequency domains were monitored.

In all subjects, standardized operative treatment was performed in the form of ETS by cutting the sympathetic chain at the level of R3 and R4 (ultrasound hook, R3, top and bottom – R4, top and bottom) and cutting the accessory Kuntz fibers. After the expert consensus from 2011, the term sympathicotomy was established for the cutting of sympathetic fibers, while the term sympathectomy means the removal of part of the sympathetic trunk [8]. Follow-up assessments were performed on the seventh day and one month after discharge from the hospital. One month after surgery, the presence and degree of CS were verified. Patients reported the presence of one of four offered degrees of CS: absent, minor, unpleasant or disabling.

Statistical methods

From the methods of descriptive statistics, the measures of central tendency were used in the study, specifically the arithmetic mean, measures of variability, namely the standard deviation and median, as well as relative numbers. From the methods of analytical statistics, methods for identifying empirical distributions (Kolmogorov–Smirnov test) were used, along with methods for assessing the significance of differences depending on the type of data and distribution, specifically the independent samples t-test, Mann–Whitney U test, and χ^2 test. To assess the significance of the relationship between predictor variables and outcome in relation to the time elapsed until the outcome, univariate and multivariate Cox regression analyses were used. The discriminatory capacity, i.e., the predictive accuracy of the examined parameters for the presence of CS, was determined by the area under the receiver operating characteristic (ROC) curve (c-statistic), and the best cut-off value of variables for predicting CS was also estimated. A c-statistic above 0.7 was considered an acceptable discriminative capacity of the model. Statistical data processing was performed in JASP software, version 0.96.0 (University of Amsterdam, Amsterdam, The Netherlands), and $p < 0.05$ was used as the criterion for statistical significance.

RESULTS

The study included 100 patients, aged 18–54 years, with an average age of 28.33 ± 8.51 years, a median age of 24 years (10/100), of whom 57 (57%) were female. There was no statistically significant difference in sex distribution in the observed groups PP and PPA PFH, $p = 0.162$.

CS was absent in 61 (61%) patients who were operated on ($p = 0.028$). Minor CS was most frequent ($p < 0.01$), and the incidence of unpleasant CS was higher than that of disabling CS ($p = 0.011$) (Figure 1).

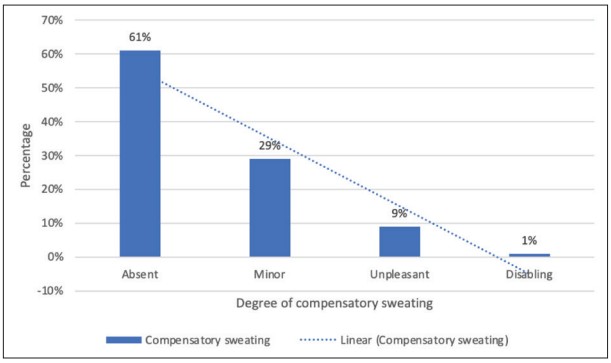


Figure 1. Frequency of different degrees of postoperative compensatory sweating

Table 1. Mean patient age and gender and incidence of postoperative compensatory sweating in relation to type of primary focal hyperhidrosis

Parameters		Type of hyperhidrosis								p
		Palmar-plantar				Palmar-plantar-axillary				
		Mean	SD	N	%	Mean	SD	N	%	
Age (years)		29.18	8.73			27.48	8.27			0.320[a]
Sex	Male			30	52.6			27	47.4	0.545[b]
	Female			20	46.5			23	53.5	
Compensatory sweating	Absent			32	52.5			29	47.5	0.666[b]
	Minor			13	44.8			16	55.2	
	Unpleasant			5	55.6			4	44.4	
	Disabling			0	0			1	100	
Compensatory sweating	No			32	52.5			29	47.5	0.539[b]
	Yes			18	46.2			21	53.8	

SD – standard deviation;
[a]Mann–Whitney U-test;
[b]χ^2 test

Table 2. Mean values of the examined heart rate variability parameters in patients with and without compensatory sweating

Heart rate variability parameters	Compensatory sweating								p
	No				Yes				
	Mean	SD	Min	Max	Mean	SD	Min	Max	
HR maximum	127.7	19	85	173	121.3	16.8	92	162	0.088[c]
HR minimum	53.9	8.5	40	77	52.7	6.6	38	70	0.466[c]
HR	74.3	9.4	55	98	72.7	9.3	49	92	0.390[c]
SDNN (ms)	144.1	31.9	86.5	229.7	146.2	28.8	99.9	232.2	0.849[c]
NN (RR)	822.6	104	598	1072	842.9	114.9	649.4	1222.5	0.363[c]
SDANN	153.1	71.3	60.1	402.9	142.8	65.8	75.9	428.6	0.377[c]
RMSSD (ms)	53.2	51.2	21	412.7	55.2	30.6	23.5	151	0.283[c]
pNN50%	20.8	30.3	2.4	236.1	19.9	12.8	1.9	57.1	0.322[c]
LF (ms^2)	1290.6	721.8	298.4	4213.5	1519.4	1037.1	359.9	3951	0.621[c]
LF normalized	68.6	9.6	47	91.7	65.3	13.8	16.1	86.2	0.300[c]
HF	532.1	359.1	98.2	1466.1	945.8	1513.9	10.7	8585.5	0.276[c]
HF normalized	27.9	9.2	12.1	51.2	31.7	19.81	4	127.9	0.527[c]
LF/HF	2.9	1.4	1	6.7	2.7	1.42	0.8	7.1	0.334[c]

HR – heart rate; RR – relative risk; SDNN – standard deviation of normal RR intervals; NN – normal RR interval; SDANN – standard deviation of all average five-minute RR intervals; RMSSD – square root of the mean value of the square of the difference of successive RR intervals; pNN50% – the proportion of adjacent normal RR intervals that differ by more than 50 ms; LF – low-frequency range of heart rate variability; HF – high-frequency range of heart rate variability;
[c]independent samples t-test

Table 3. Mean age and frequency by sex in patients with and without compensatory sweating

Parameters		Compensatory sweating								p
		No				Yes				
	Mean	SD	N	%	Mean	SD	N	%		
Age (years)		25.61	6.6			32.59	9.45			< 0.01[a]
Sex	Male			35	61.4			22	38.6	0.924[b]
	Female			26	60.5			17	39.5	

[a]Mann–Whitney U test;
[b]χ^2 test

There were no statistically significant differences in the mean patient age ($p < 0.32$), or in sex distribution and the frequency of CS in relation to the type of PFH ($p > 0.05$) (Table 1).

There was no statistically significant difference in the mean values of the examined preoperative HRV parameters in patients with and without CS ($p > 0.05$) (Table 2).

Patients with CS were statistically significantly older ($p < 0.01$). There was no statistically significant difference in the sex distribution in patients with and without CS (Table 3).

In the group of patients with LF/HF < 2, there was no statistically significant difference in the mean values of preoperative LF/HF (1.59 *vs.* 1.44; p = 0.163), or in the group of patients with LF/HF ≥ 2 (3.41 *vs.* 3.5; p = 0.801). In the group of patients with PP PFH and LF/HF < 2, there was no statistically significant difference in mean LF/HF values (1.66 *vs.* 1.38; p = 0.078), or in the group of patients with LF/HF ≥ 2 (3.24 *vs.* 3.33; p = 0.851). In the group of patients with PPA PFH and LF/HF < 2, there was no statistically significant difference in the mean values of preoperative LF/HF (1.54 *vs.* 1.51; p = 0.876), or in the group of patients with LF/HF ≥ 2 (3.63 *vs.* 3.61; p = 0.974) (Figure 2).

Univariate Cox regression analysis showed that univariate statistically significant predictors of postoperative CS were age ($p < 0.01$) and HF after statistical control for age ($p < 0.01$) (Table 4).

Multivariate Cox regression analysis showed that age [relative risk (RR) 1.11; 95% confidence interval (CI) (1.05–1.18); $p < 0.01$] and HF before ETS [RR 1.00; 95% CI (1.00–1.001), $p < 0.01$] were independent predictors of CS. The analysis of ROC curves for the patient age variable showed that it has a statistically significant predictive accuracy for the occurrence of HF (area under the ROC curve 0.7333; 95% CI (0.634–0.833); $p < 0.01$), while it was not shown for HF. On that occasion, the best threshold value of the patient's age for predicting CS after ETS was determined, which is 23.5 years, with a combination of sensitivity 0.82 and specificity 0.47.

DISCUSSION

CS as the most frequent side effect of ETS was present in 39% of subjects (39/100), whereby it was characterized as unpleasant by 9% (9/100) and disabling by 1% (1/100). The literature reports a remarkably wide range in the prevalence of CS, varying from 3% to 98%. CS has been identified as the main culprit for patient dissatisfaction after ETS [4]. Such a large incidence range was a consequence of the unclassified nomenclature, as well as the definition of CS itself. In some studies, CS was reported exclusively when it reached incapacitating levels, while others documented even a minimal subjective feeling of increased sweating [5, 18, 19]. Many studies have indicated that the degree of CS is directly proportional to the height of the transection level of the sympathetic trunk, with a note that the degree of CS is the highest with R2 transection [18, 19]. Our study was designed so that all sympathetic chain transections were performed at the R3–R4 level precisely to avoid an increased incidence of CS.

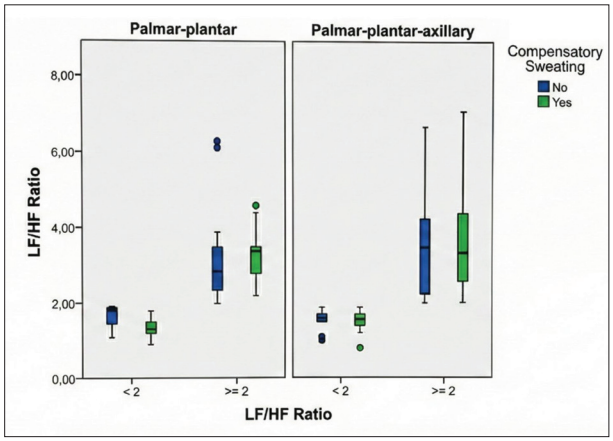


Figure 2. Mean values of LF/HF ratio in groups according to the presence of compensatory sweating, in patients with different types of hyperhidrosis; LF – low-frequency range of heart rate variability; HF – high-frequency range of heart rate variability

Table 4. Univariate Cox regression for the prediction of compensatory sweating, controlled for age

Variable	RR	95% CI	p
Age (years)	1.11	1.05–1.18	< 0.01[d]
Gender	1.04	0.46–2.34	0.92[d]
HR maximum	0.99	0.97–1.01	0.32[d]
HR minimum	0.97	0.94–1.01	0.15[d]
HR	0.99	0.94–1.04	0.67[d]
SDNN (ms)	1.01	0.99–1.02	0.11[d]
SDNN (125–149) (ms)	2.307	0.99–5.71	0.07[d]
SDNN (> 150) pre (ms)	2.069	0.84–5.11	1.15[d]
NN (RR)	1.00	0.99–1.01	0.14[d]
SDANN	1.00	0.99–1.01	0.59[d]
RMSSD (ms)	0.82	0.99–1.01	0.82[d]
pNN50%	0.99	0.98–1.01	0.91[d]
LF (ms^2)	1.00	1.00–1.00	0.07[d]
LF normalized	0.98	0.96–1.01	0.23[d]
HF	1.00	1.00–1.01	< 0.01[d]
HF normalized	1.01	0.99–1.03	0.08[d]
LF/HF	0.97	0.75–1.23	0.77[d]

HR – heart rate; RR – relative risk; SDNN – standard deviation of normal RR intervals; NN – normal RR interval; SDANN – standard deviation of all average five-minute RR intervals; RMSSD – the square root of the mean value of the square of the difference of successive RR intervals; pNN50% – the proportion of adjacent normal RR intervals that differ by more than 50 ms; LF – low-frequency range of heart rate variability; HF – high-frequency range of heart rate variability; CI – confidence interval;
[d]univariate Cox regression

We believe that the degree of CS in our study is at a satisfactory level, which is primarily a consequence of the standardized operative technique, suitable selection of patients for ETS and detailed preoperative information provided to patients about potential side effects and complications. It is interesting that the only patient with a disabling form of CS was a 37-year-old male (recommended age for CS prevention is 25 years) and that he suffered from malaria in childhood.

An easily available, non-invasive predictor of CS would greatly improve patient selection and the success of ETS. Hyperstimulated sympathetic fibers responsible for sweating also partially innervate the heart. It makes sense to examine the potential use of HRV as a diagnostic method for the prediction of CS. There are three types of HRV measurement: ultrashort (under five minutes), short (five minutes), and 24-hour measurement. These measurements cannot be compared with each other due to different influences on the 24-hour measurement, which best reflects the real situation [20, 21]. This is why we have used the 24-hour measurement of HRV.

The LF/HF ratio has been proposed as an indicator of sympathovagal balance, with values of 0.5–2. Values over two units are considered a consequence of pathological SNS stimulation or parasympathetic nervous system (PNS) inactivity, while values below 0.5 are a consequence of pathological PNS stimulation [12, 20, 22]. We singled out LF/HF as a parameter to determine whether CS is more common in the group with sympathetic predominance (LF/HF ≥ 2). It was determined that there is no difference in the occurrence of CS in relation to LF/HF values. The difference did not exist even when this group was divided into PP and PPA PFH. The lowest measured value of LF/HF in our study was 0.9. Consequently, not a single patient had parasympathetic predominance, as expected, but all were either in balance or in the zone of sympathetic predominance.

After the analysis of the HRV parameters to predict the occurrence of CS, the existence of a statistically significant correlation of HF with CS was established. However, after ROC curve testing, significance was not obtained. We identified only two studies that correlated HRV and CS. Hyun et al. [23] do not report any HRV parameter as a possible predictor of CS. Tiwari et al. [24] report HF as a positive predictive factor for CS. Unlike our study, HF prediction was not tested on ROC curves. Both studies were performed with five-minute HRV recordings, which is a major limitation.

Although none of the HRV parameters in our study can be used to predict CS, we confirmed that CS is correlated with age and that patients below 23 years of age have a lower probability of CS. In current recommendations, an age below 25 years is considered appropriate for ETS [3, 5, 8].

CONCLUSION

HRV parameters cannot be used as predictors for the occurrence of CS after bilateral thoracoscopic sympathicotomy in persons suffering from PFH. Although the HF parameter was singled out in our study as a potential predictive factor, after ROC curve testing, significance was not obtained. Our study confirmed that the optimal age at which to perform bilateral thoracoscopic sympathicotomy is below 25 years, more precisely below 23.5 years.

Ethics: The study was approved by the Ethics Commission of the Institute for Pulmonary Diseases of Vojvodina under the number 6-VI/1 on December 9, 2021, and was conducted in accordance with the principles of the Declaration of Helsinki.

Conflict of interest: None declared.

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Да ли се варијабилност срчане фреквенције може користити за предвиђање компензаторног знојења након ендоскопске торакалне симпатикотомије код болесника са примарном фокалном хиперхидрозом?

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САЖЕТАК

Увод/Циљ Хиперхидроза је стање патолошког прекомерног знојења које превазилази физиолошке потребе организма за терморегулацијом. Ендоскопска торакална симпатикотомија (ЕТС) представља уобичајен начин лечења примарне фокалне хиперхидрозе (ПФХ), али може довести до појаве компензаторног знојења (КЗ), које представља њену најчешћу постоперативну компликацију. Циљ ове студије био је да се испита да ли параметри варијабилности срчане фреквенције могу предвидети појаву КЗ.

Методе Спроведена је проспективна једноцентрична студија која је обухватила 100 болесника са ПФХ који су подвргнути ЕТС. Преоперативно је код свих болесника урађен 24-часовни холтер ЕКГ ради процене стандардних параметара варијабилности срчане фреквенције. ЕТС је изведена пресецањем симпатичког ланца на нивоу трећег (*R*3) и четвртог ребра (*R*4), укључујући и помоћна Кунцова влакна. Месец дана након операције процењивани су присуство и степен КЗ.

Резултати КЗ није било присутно код 61% болесника ($p = 0,028$). Благо КЗ било је најчешћи облик ($p < 0,01$). Није утврђена статистички значајна разлика у анализираним преоперативним параметрима варијабилности срчане фреквенције између болесника са КЗ и без њега ($p > 0,05$), укључујући категорије односа *LF/HF*, које представљају уравнотежену аутономну активност (*LF/HF* < 2) и симпатичку доминацију (*LF/HF* ≥ 2). Анализа *ROC* криве показала је да старост има статистички значајну предиктивну тачност за појаву КЗ (*AUC* 0,7333; 95% *CI* 0,634–0,833; $p < 0,01$), са оптималном граничном вредношћу од 23,5 година (сензитивност 0,82; специфичност 0,47).

Закључак Параметри варијабилности срчане фреквенције не могу поуздано предвидети појаву КЗ након билатералне ЕТС код болесника са ПФХ. Студија је потврдила да је оптимална старост за извођење операције испод 23,5 године.

Кључне речи: видео-асистирана торакална хирургија; знојење; симпатектомија; аутономни нервни систем; минимално инвазивна хирургија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Functional results in surgical treatment of unstable pertrochanteric fractures (AO/OTA A2) treated by TRIGEN INTERTAN and DHS methods

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SUMMARY

Introduction/Objective Intertrochanteric fractures of the femur are one of the biggest public health problems. This paper aims to determine the functional results and features of operative treatment of unstable intertrochanteric femoral fractures.

Methods This retrospective study included 67 patients who were treated surgically for AO/OTA A2 pertrochanteric fractures at the Department of Orthopedic Surgery and Traumatology of the Kosovska Mitrovica Clinical Hospital Center and at the Academician Prof. Dr. Milorad Mitković Clinic for Orthopedic Surgery and Traumatology of the Niš University Clinical Center. There were two groups of patients regarding the fixation method used – the dynamic hip screw (DHS) group and the TRIGEN INTERTAN group.

Results Patients treated using the DHS method had a statistically confirmed longer operation time, greater intraoperative blood loss, and longer hospitalization in relation to patients treated using the TRIGEN INTERTAN method ($p < 0.001$). In contrast, patients from the TRIGEN INTERTAN group spent less time in the intensive care unit and had more favorable radiological findings regarding fracture healing, as well as better functional outcomes assessed by the Harris Hip Score and Barthel index, which were also statistically significant. The peak-to-peak tip–apex distance was greater in the DHS group, with a significant difference between the techniques ($p = 0.019$). According to the Charlson Comorbidity Index, patients who were treated using the DHS method had more pronounced comorbidities ($p = 0.022$).

Conclusion Surgical treatment of unstable pertrochanteric fractures using intramedullary fixation with TRIGEN INTERTAN is very safe and effective in all patients, especially in elderly patients with comorbidities.

Keywords: unstable; pertrochanteric; fractures; hip function

INTRODUCTION

Trochanteric fractures of the femur represent a significant public health problem. They can result in disability, reduced quality of life, and increased incidence of mortality. The incidence of intertrochanteric fractures is increasing due to the growth of the elderly population as a result of improved living standards and advances in modern medicine. It is estimated that 6.25 million people worldwide will sustain trochanteric fractures by the year 2050 [1].

Trochanteric fractures belong to the group of extra-articular fractures and are one of the most common injuries in the elderly population, with approximately 90% occurring in patients over the age of 65. The treatment of trochanteric fractures is often challenging due to the complexity of the fracture, the difficulty in achieving anatomical reduction and stable fixation, and the comorbidities of the patient. Elderly patients with osteoporosis often have unstable trochanteric fractures caused by a minor external force. Conversely, in younger people, intertrochanteric fractures mostly occur as a result of high-energy force [2].

The AO/OTA classification is the most widely used classification of bone fractures, dividing the trochanteric fractures (31-A) into three main groups, which are further subdivided into nine subtypes (Figure 1). Fracture type 31-A1 includes simple two-part fractures, while 31-A2 includes a separated lesser trochanter, without (31-A2.1) or with additional comminution in the intertrochanteric region (31-A2.2 and 31-A2.3). Fractures of type 31-A3 pass through the lateral femoral wall – defined as the lateral cortex distal to the greater trochanter (distal to the femoral ridge) [3]. A1 fractures are considered stable, while A2 and A3 are considered unstable trochanteric fractures. The AO/OTA classification distinguishes between pertrochanteric (A1 and A2) and intertrochanteric fractures (A3).

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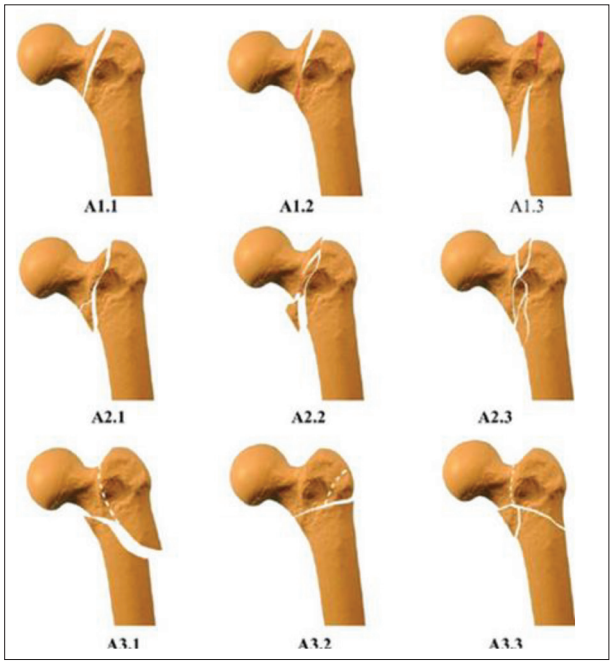


Figure 1. *Arbeitsgemeinschaft für Osteosynthesefragen* (AO) classification of intertrochanteric fractures

Internal fixation of trochanteric fractures can be extramedullary (dynamic hip screw (DHS), Selfdynamizable Internal Fixator, etc.) and intramedullary (Gamma Nail, TRIGEN INTERTAN, proximal femoral nail antirotation, etc.) [4, 5].

The aim of this paper was to determine the functional results and features of surgical treatment of unstable pertrochanteric fractures using DHS and the TRIGEN INTERTAN short cephalomedullary nail.

METHODS

The research was performed as a retrospective study and included 67 patients with unstable pertrochanteric fractures that were treated surgically 2015–2022 at the Department of Orthopedic Surgery and Traumatology of the Kosovska Mitrovica Clinical Hospital Center (CHC) and at the Academician Prof. Dr. Milorad Mitković Clinic for Orthopedic Surgery and Traumatology of the Niš University Clinical Center. There were two groups of patients regarding the fixation method used – the DHS group and the TRIGEN INTERTAN group.

The Singh osteoporosis index was used to determine the degree of osteoporosis. It consists of six groups (the main role in the gradation of the degrees of osteoporosis is played by the interruption of the main tension trabeculae): grade 6 (all trabecular groups are present), grade 5 (the main tension and main compression groups are emphasized), grade 4 (the main tension trabeculae are reduced), grade 3 (the main tension trabeculae are interrupted opposite the greater trochanter. This grade indicates definitive osteoporosis), grade 2 (the presence of only the main compression trabeculae), grade 1 (the main compression trabeculae are reduced).

Postoperative X-ray follow-up was used to assess the postoperative collodiaphyseal angle and tip–apex distance (TAD).

The Harris Hip Score (HHS), as a commonly used score to assess hip function, was measured over a period of six to 12 months to represent long-term functional outcomes. HHS is a rating scale with a total of 100 points (excellent, ≥ 90 points; very good, 80–89 points; good, 70–79 points; and poor, < 70 points). This score includes pain, movement, deformity, and function.

The Barthel scale/index is a scale that was used to measure performance in activities of daily living (ADL). Ten variables describing ADL and mobility are scored, with higher scores reflecting greater ability to function independently six months after discharge from the hospital. We have classified patients into five groups: 0–4 = complete dependence; 5–12 = severe dependence; 13–18 = moderate dependence; 19 = slight dependence.

Based on the Charlson Comorbidity Index (CCI) score, the severity of comorbidity was categorized into three grades: 1. mild (CCI scores of 1–2); 2. moderate (CCI scores of 3–4); 3. severe (CCI scores ≥ 5). It should also be noted that the callus was analyzed on the radiological image. Native X-rays were used for the analysis.

Also, it should be emphasized that intraoperative blood loss was determined by the level of aspirated fluid in the reservoir.

Statistical analysis

The data collected in the study were analyzed using IBM SPSS Statistics 20 software. Descriptive parameters were expressed as frequencies (n) and percentages (%). Comparisons were performed using the χ^2 test for categorical data, and the Mann–Whitney U test for continuous data. Statistical significance was set at $p < 0.05$.

RESULTS

Descriptive data, including general data, clinical, radiological, and functional parameters, are presented in Table 1.

Table 1. Baseline demographic and clinical characteristics among the groups

Variables		Groups		p
		TRIGEN INTERTAN [n (%)]	DHS [n (%)]	
Sex	Male	10 (22.7%)	6 (26.1%)	0.759
	Female	34 (77.3%)	17 (17.5%)	
Age	40–60 years	3 (6.8%)	0	0.382
	61–80 years	17 (38.6%)	8 (34.8%)	
	> 80 years	24 (54.5%)	15 (65.2%)	
AO/OTA fracture type	A2.1	2 (4.5%)	8 (34.8%)	< 0.001
	A2.2	26 (59.1%)	15 (65.2%)	
	A2.3	16 (36.4%)	0	

AO – *Arbeitsgemeinschaft für Osteosynthesefragen*; OTA – Orthopaedic Trauma Association

The results indicated that there was no statistically significant association between the type of surgery and patient gender, age, or the degree of osteoporosis. However, a significant difference between the surgical method groups was observed regarding blood transfusion – all transfusions were administered to patients treated with DHS, indicating greater intraoperative or postoperative blood loss in these patients. Furthermore, the type of surgical technique was significantly associated with the incidence of long-term postoperative complications. Patients treated with the TRIGEN INTERTAN method experienced a lower complication rate, whereas those who were treated by DHS had a higher rate of adverse outcomes, delayed healing, bone infections, and cut-out (Table 2).

Patients treated with the DHS technique had a statistically significantly longer duration of surgery, greater intraoperative blood loss and longer hospitalization than patients treated with the TRIGEN INTERTAN method ($p < 0.001$). In contrast, patients from the TRIGEN INTERTAN group had more favorable radiological findings regarding fracture healing, as well as better functional outcomes assessed by the HHS and Barthel index, which were also statistically significant. The peak-to-peak TAD was greater in the DHS group, with a significant difference between the techniques ($p = 0.019$) (Table 3).

According to the CCI, patients who were operated on using the DHS technique had more pronounced comorbidities ($p = 0.022$).

Examination of the relationship between the type of surgical method and AO/OTA fracture types revealed a statistically significant correlation ($p < 0.001$). The results confirm that in A2.2 and A2.3 fractures, surgeons more often perform fixation with the TRIGEN INTERTAN implant (Table 3).

Table 2. Perioperative characteristics and postoperative outcomes in all patients

Variable		n (%)
Sex	Male	16 (23.9%)
	Female	51 (76.1%)
AO/OTA	A2.1	10 (14.9%)
	A2.2	41 (61.2%)
	A2.3	16 (23.9%)
Fixation method	TRIGEN INTERTAN	44 (65.7%)
	DHS	23 (34.3%)
Blood transfusion	No transfusion	44 (65.7%)
	350 ml	17 (25.4%)
	700 ml	6 (9%)
Age	40–60 years	3 (4.5%)
	61–80 years	25 (37.13%)
	> 80 years	39 (58.2%)
ICU stay	No ICU	29 (43.3%)
	< 5 days	0
	≥ 5 days	38 (56.7%)
TAD	< 20 mm	8 (11.9%)
	21–30 mm	52 (77.6%)
	> 30 mm	7 (10.4%)
CCI	5–6	15 (22.4%)
	2–4	51 (76.1%)
	0–1	1 (1.5%)
Singh score of osteoporosis	Severe osteoporosis	0
	Moderate osteoporosis	4 (6%)
	Severe osteopenia	14 (20.9%)
	Moderate osteopenia	30 (44.8%)
	Mild osteopenia	17 (25.4%)
	Normal bone	2 (3%)
Long-term complications	Nonunion	2 (3%)
	Malunion	4 (6%)
	Bone infection	2 (3%)
	Refracture	0
	Cut-out	4 (6%)
	No complication	55 (82%)
HHS	< 70	0
	71–80	0
	81–90	18 (26.9%)
	91–100	49 (73.1%)
Operation time	< 30 min	8 (11.9%)
	31–60 min	41 (61.2%)
	61–90 min	18 (26.9%)
Dependence according to the Barthel index	Total dependence	0
	Severe dependence	8 (11.9%)
	Moderate dependence	17 (25.4%)
	Mild dependence / functional independence	42 (62.7%)
Intraoperative blood loss	< 100 ml	36 (53.7%)
	100–350 ml	31 (46.3%)
	> 350 ml	0
Radiologically visible callus at 24 weeks	Adequate mature callus visible	33 (49.3%)
	Visible immature callus	31 (46.3%)
	No visible callus	3 (4.5%)

TAD – tip–apex distance; CCI – Charlson Comorbidity Index; AO – *Arbeitsgemeinschaft für Osteosynthesefragen*; OTA – Orthopaedic Trauma Association; HHS – Harris Hip Score; ICU – intensive care unit

DISCUSSION

These findings emphasize the clinical importance of selecting an appropriate surgical technique, particularly in relation to blood transfusion requirements and the prevention of long-term complications. In contrast, factors such as gender, age, and the degree of osteoporosis did not significantly influence the choice of surgical intervention.

The mean age of all patients was 73.58 ± 15.86 years, ranging 62–97 years. A significant proportion of patients, 39 out of 67 (58.2%), were older than 80 years. The results of our study are in line with several other studies [6, 7].

In our study, female patients accounted for 76.1% of all cases. This result is consistent with Krishna et al. [8]. In our study, it was found that A2.2 pertrochanteric fractures were the most common, accounting for 61.2%, while A2.1 and A2.3 fractures were less common. These results are in line with those of Murugan et al. [9]. In older people, trochanteric fractures usually occur as a result of a fall, most often with an indirect impact on the hip. Although the traumatic force is of low intensity, most of these fractures are multi-fragmentary and complex due to osteoporotic changes in the skeletal system at various stages. Our study

Table 3. Perioperative characteristics and postoperative outcomes among the groups

Variables		Groups		p
		TRIGEN INTERTAN [n (%)]	DHS [n (%)]	
Singh score of osteoporosis	Severe osteoporosis	0	0	0.779
	Moderate osteoporosis	2 (4.5%)	2 (8.7%)	
	Severe osteopenia	10 (22.7%)	4 (17.4%)	
	Moderate osteopenia	19 (43.2%)	11 (47.8%)	
	Mild osteopenia	11 (25%)	6 (26.1%)	
	Normal bone	2 (4.5%)	0	
CCI	5–6	14 (31.8%)	1 (4.3%)	0.022
	2–4	29 (65.9%)	22 (95.7%)	
	0–1	1 (2.3%)	0	
Length of hospital stay	7–10 days	40 (90.9%)	2 (8.7%)	< 0.001
	11–15 days	4 (9.1%)	21 (91.3%)	
Operation time	≤ 30 min	8 (18.2%)	0	< 0.001
	31–60 min	36 (81.8%)	5 (21.7%)	
	61–90 min	0	18 (78.3%)	
Intraoperative blood loss	≤ 100 ml	32 (72.7%)	4 (17.4%)	< 0.001
	> 100 ml (up to 350 ml)	12 (27.3%)	19 (82.6%)	
Blood transfusion	No	44 (100%)	0	< 0.001
	350 ml	0	6 (26.1%)	
	700 ml	0	17 (73.9%)	
ICU stay	No ICU stay	26 (59.1%)	3 (13%)	< 0.001
	< 5 days	18 (40.9%)	20 (87%)	
TAD	< 20 mm	7 (15.9%)	1 (4.3%)	0.019
	20–30 mm	35 (79.5%)	17 (73.9%)	
	> 30 mm	2 (4.5%)	5 (21.7%)	
Long-term complications	Nonunion	0	2 (8.7%)	0.003
	Malunion	1 (2.3%)	3 (13%)	
	Bone infection	0	2 (8.7%)	
	Cut-out	1 (2.3%)	3 (13%)	
	No complication	42 (95.5%)	13 (56.5%)	
HHS	81–90	4 (9.1%)	14 (60.9%)	< 0.001
	91–100	40 (90.9%)	9 (39.1%)	
Barthel Index	Total dependence	0	0	< 0.001
	Severe dependence	0	8 (34.8%)	
	Moderate dependence	5 (11.4%)	12 (52.2%)	
	Mild dependence	39 (88.6%)	3 (13%)	
Radiologically visible callus at 24 weeks	Adequate mature callus visible	27 (61.4%)	6 (26.1%)	0.005
	Immature callus visible	16 (36.4%)	15 (65.2%)	
	No visible callus	1 (2.3%)	2 (8.7%)	

TAD – tip–apex distance; CCI – Charlson Comorbidity Index; HHS – Harris Hip Score; ICU – intensive care unit

also found that type A2.2 fractures were more common in female patients (45.7%), while A2.3 pertrochanteric fractures were more common in cases resulting from high-intensity force. Thus, it could be considered that A2.1 and A2.2 pertrochanteric fractures occurred more frequently in cases of low-intensity force.

In our study, we observed that 95.5% of all patients achieved fracture union. This union rate is in line with findings from other studies conducted by Yang et al. [10] and Wang et al. [11]. This high rate of union can be attributed primarily to the rich blood supply in the trochanteric area. Mature callus in the DHS group was radiologically visible in only 65.9% of the subjects. It should be noted that the formation of radiologically visible mature bone callus 24 weeks after surgery was achieved more frequently with INTERTAN than with DHS, which would support the generally faster callus maturation with the INTERTAN method.

All 23 patients in the DHS group had received blood transfusion during surgery with a mean volume of 350 ml. Patients treated with TRIGEN INTERTAN did not receive transfusions. These results are consistent with the studies of Wang et al. [11] and Kassem et al. [12].

The patients included in our study didn't have complications such as device failure or device malfunction. Regarding late complications, 56.5% of all cases had some kind of postoperative complications (deep infection, varus collapse, nonunion, malunion). The complication rate was higher in the DHS group ($p < 0.05$) and it should be emphasized that the proportion of late complications in this study was higher with DHS, probably due to the use of DHS in pertrochanteric fractures with pronounced comminution (A2.2). The large freedom of lag screw translation in DHS may be a factor for later complications in A2.2 fractures. In contrast to DHS, there are extramedullary methods that have limited lag screw translation, as well as an additional anti-rotation or additional screw (which DHS does not have), as is the case with the Selfdynamizable Internal Fixator, and it could be considered that not all extramedullary methods will produce the same results as DHS in these fractures. The immense significance of the anti-rotational screw in the Selfdynamizable Internal Fixator lies in its ability to achieve rotational stability, which serves as an excellent predictor of faster and superior fracture healing, particularly in these types of fractures that are inherently highly unstable. This finding aligns with the conclusions of studies of Ćirić et al. [13] and Mitković et al. [14]. DHS has only one lag screw and is more rotationally unstable compared to extramedullary methods that include two lag screws or one lag screw and one additional femoral neck anti-rotation pin. These results match those of Rashid et al. [15] and Shin et al. [16].

It should be noted that all patients survived the six-month postoperative period.

The quality of life after surgery for a pertrochanteric fracture depends on the degree of hip function recovery. Compared to DHS, there was no statistically significant difference between the two groups. However, in patients with a trochanteric fracture, walking ability represents an important outcome. It is widely considered that partial weight bearing during walking can begin earlier after intramedullary fixation than after fixation with DHS. In our

study, most patients in the TRIGEN INTERTAN group had an excellent score, while in the DHS group most patients had a good HHS. Such a large difference could be influenced by the larger proportion of patients with comorbidities in the DHS group. Earlier weight bearing on the operated leg reduces the risk of some complications, such as decubitus ulcers, thromboembolism, and pneumonia. Our results are in correlation with the study of Kang et al. [17], who presented that most patients (140/168) in the intramedullary group could start walking with crutches or a walker within one week after surgery.

Our study showed that weight bearing on the operated leg after fixation of a trochanteric fracture can generally be initiated earlier when using TRIGEN INTERTAN than when using DHS.

Functional independence was significantly higher in the TRIGEN INTERTAN group (88.6%) compared to the DHS group (13%). This difference could be influenced by the larger proportion of patients with comorbidities in the DHS group. So, we can safely say it is in complete correlation with published data such as those of Smith et al. [18].

The peak-to-peak TAD values were generally higher in the DHS group compared to the INTERTAN group, which is most likely why there were more cut-outs in the DHS group compared to the INTERTAN group, and thus this study confirmed the importance of better intraoperative TAD in the occurrence of cut-outs. Comparing the results with the official literature and research conducted by Luo et al. [19], we find that the results of these two studies are in correlation with each other.

Regarding mature callus visibility 24 weeks after surgery, we found that adequate mature callus was more often seen radiologically in patients from the TRIGEN INTERTAN group (61.4%) compared to the DHS group (26.1%). This finding is in accordance with Zhou et al. [20], who found adequate mature callus to be visible in 65% of patients with intramedullary fixation. Generally greater development of callus in patients in the INTERTAN group can be influenced by the rotatory stability of the proximal fragment of the pertrochanteric fracture, which is less when using DHS [20]. These results indicate a clinically relevant difference in the course and outcome of treatment depending on the chosen operative method.

CONCLUSION

In conclusion, it could be stated that the surgical treatment of unstable pertrochanteric fractures by TRIGEN INTERTAN can be followed by a lower need for blood transfusion, earlier weight bearing on the operated leg, higher hip function, and earlier radiological callus visibility, in relation to the treatment by DHS. However, it should be noted that the final outcomes may also be affected by the level of comorbidity and intraoperative TAD.

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Ethics: In order to protect patient data, all data were encrypted and processed in accordance with the ethical principles of privacy protection and based on the decisions of the Ethics Committee of the Kosovska Mitrovica CHC, No. 1039/25.02.2023 as well as the Ethics Committee of the Niš UCC, No. 7380/15.03.2023.

Conflict of interest: None declared.

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Функционални резултати хируршког лечења нестабилних пертрохантерних прелома (AO/OTA A2) методама *TRIGEN INTERTAN* и *DHS*

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САЖЕТАК

Увод/Циљ Интертрохантерни преломи фемура један су од највећих јавноздравствених проблема. Циљ овог рада био је да се утврде функционални резултати и карактеристике оперативног лечења нестабилних интертрохантерних прелома фемура.

Методе Истраживање је спроведено као ретроспективна студија која је обухватила 67 болесника оперативно лечених од 2015. године на Одељењу за ортопедску хирургију и трауматологију Клиничко-болничког центра Косовска Митровица и на Клиници за ортопедску хирургију и трауматологију „Академик проф. др Милорад Митковић" Универзитетског клиничког центра Ниш. Болесници су, према примењеној методи фиксације, били подељени у две групе – групу *DHS* (*Dynamic Hip Screw*) и групу *TRIGEN INTERTAN*.

Резултати Болесници лечени техником *DHS* имали су статистички значајно дуже трајање операције, већи интраоперативни губитак крви и дужу хоспитализацију него болесници лечени методом *TRIGEN INTERTAN* (сви $p < 0,001$). Насупрот томе, болесници из групе *TRIGEN INTERTAN* нису боравили на одељењу интензивне неге, али су имали повољније радиолошке налазе у погледу зарастања прелома, као и боље функционалне исходе процењене Харисовим скором за кук (*Harris Hip Score*) и Бартеловим индексом (*Barthel Index*), што је такође било статистички значајно. Вредност растојања врх–апекс (*tip-apex distance*) била је већа у групи *DHS*, са статистички значајном разликом између техника ($p = 0,019$). Према Чарлсоновом индексу коморбидитета (*Charlson Comorbidity Index*), болесници који су оперисани техником *DHS* имали су израженије коморбидитете ($p = 0,022$).

Закључак Оперативно лечење нестабилних интертрохантерних прелома интрамедуларном фиксацијом методом *TRIGEN INTERTAN* веома је безбедно и ефикасно код свих болесника, посебно код старијих болесника са коморбидитетима.

Кључне речи: нестабилни; пертрохантерни; преломи; функција кука

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Clinical, functional, and radiographic outcomes of primary total knee arthroplasty using a rotating hinge implant – a retrospective cohort study

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SUMMARY

Introduction/Objective The aim was to evaluate the clinical, functional, and radiographic outcomes, as well as implant survivorship, of primary total knee arthroplasty using a rotating hinge knee prosthesis in complex primary knees.

Methods This single-center, retrospective observational cohort study included 32 patients (32 knees) operated between December 1, 2023, and December 31, 2025. Primary outcomes were cumulative implant survivorship (Kaplan–Meier analysis with revision for any cause as the endpoint) and complication rates. Secondary outcomes included functional scores using the Oxford Knee Score (OKS), range of motion, coronal alignment correction via the mechanical femorotibial angle, and radiographic stability.

Results The median follow-up was 20.3 months. Surgical indications included primary (43.8%) and secondary osteoarthritis (21.9%), post-traumatic arthritis (21.9%), neuropathic arthropathy (9.4%), and proximal tibial nonunion (3.1%). Preoperative ligamentous instability was present in 87.5% of knees. Cumulative 20-month implant survivorship was 87.5% (all four revisions occurred within five months postoperatively due to early periprosthetic joint infection and wound complications; 0% aseptic loosening). The overall complication rate was 34.4%. Postoperatively, the mean OKS significantly increased from 13.25 ± 5.62 to 41.09 ± 3.74 (ΔOKS = +27.84, $p < 0.001$), median range of motion increased from 70° to 115° ($p < 0.001$), and absolute coronal deformity (deviation from neutral biomechanical axis) decreased from 23.9° to 2.36° ($p < 0.001$).

Conclusion Primary total knee arthroplasty with a rotating hinge knee implant provides excellent functional recovery, robust early mechanical stability, and predictable deformity correction in complex primary cases, though strict infection control and soft-tissue handling remain essential.

Keywords: total knee arthroplasty; rotating hinge knee; primary arthroplasty; survival analysis; functional outcome

INTRODUCTION

Total knee arthroplasty (TKA) represents one of the most successful and frequently performed surgical procedures in modern orthopedic surgery. The primary objective of this intervention is pain reduction, correction of deformity, restoration of joint function, and improvement in quality of life for patients with end-stage degenerative joint disease. The most common indication for TKA is advanced primary osteoarthritis, but the procedure is also increasingly utilized in inflammatory arthropathies, post-traumatic arthritis, avascular necrosis, and other conditions leading to joint destruction. Advances in implant design, refinements in surgical technique, optimized perioperative care, and standardized rehabilitation protocols have significantly contributed to the reliability and durability of TKA. In modern literature, TKA is universally recognized as a procedure that yields substantial functional recovery in the vast majority of cases [1, 2, 3].

Alongside objective clinical and radiographic parameters, patient-reported outcome measures have emerged as essential indicators of therapeutic success and functional recovery following primary TKA [1–4]. Validated joint-specific instruments, such as the Oxford Knee Score (OKS), provide direct insight into postoperative pain relief, joint function, and the restoration of daily activities from the patient's perspective [1, 2, 3]. Nevertheless, the magnitude of functional improvement varies depending on preoperative deformity severity, baseline pain intensity, functional status, medical comorbidities, and postoperative complications [1–5]. Suboptimal outcomes are most frequently linked to persistent pain, stiffness, joint instability, or complications requiring secondary surgical intervention [1, 2, 5]. Consequently, meticulous preoperative evaluation, realistic patient counseling, and individualized surgical planning are paramount when managing complex knee pathology [3, 5].

The success of primary TKA relies not only on implant selection but also on precise

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mechanical axis alignment, soft-tissue balancing, and joint stability throughout the full arc of motion [6, 7, 8]. In standard primary cases, unconstrained implants (such as posterior-stabilized or cruciate-retaining designs) are routinely employed to approximate physiological kinematics. However, a distinct subset of patients presents with complex primary pathology [6, 7, 8]. This challenging cohort includes patients with severe fixed *varus* or *valgus* deformities, gross collateral ligament insufficiency, massive bone loss, post-traumatic structural alterations, neuromuscular disorders, ankylosis, or joint hyperlaxity [6, 7, 8]. In these scenarios, standard unconstrained implants fail to provide adequate primary stability and predictable outcomes [6, 7, 8]. Collateral ligament incompetence cannot be addressed solely by soft-tissue releases, forcing the surgeon to select an implant constraint level that matches the anatomical and functional severity of the defect to compensate for irreversibly compromised soft-tissue envelope [6, 7, 8]. Inadequate constraint predisposes to catastrophic postoperative instability, whereas excessive constraint transfers increased mechanical stresses to the bone-implant or bone-cement interfaces [8, 9, 10]. Therefore, implant selection represents a critical determinant of surgical success in complex primary TKA [6–10].

Rotating hinge knee (RHK) prostheses were specifically developed to provide mechanical stability when native joint stabilizers are severely compromised [6–10]. Unlike early fixed-hinge mechanisms, modern RHK systems incorporate a mobile-bearing rotational axis that allows controlled axial rotation alongside flexion and extension [8, 9, 10]. The rotational mechanism is designed to reduce torsional stress transmission to the fixation interfaces compared with fixed-hinge designs [8, 9, 10]. In primary TKA, the application of an RHK implant is non-routine and strictly reserved for well-defined, complex clinical indications [6–10]. Most common indications comprise gross ligamentous instability, severe uncorrectable coronal deformities, massive flexion-extension gap mismatch, severe bone loss, and conditions where stability cannot be achieved with less constrained implants [6–10]. In such patients, an RHK construct facilitates safe structural reconstruction, adequate immediate stability, and early functional mobilization [6–10]. This is particularly advantageous in elderly or frail patients with poor soft-tissue quality, in whom postoperative instability would carry devastating clinical consequences [6, 7, 8]. However, because RHK implants represent the highest degree of constraint, their use requires strict adherence to surgical indications [8, 9, 10]. Potential disadvantages – such as increased stress transfer, risk of periprosthetic joint infection (PJI), aseptic loosening, and technically demanding future revisions – must be carefully weighed [8, 9, 10].

Recent literature demonstrates that when utilized in appropriately selected primary cases, modern RHK implants achieve favorable mid-term survivorship and remarkable clinical improvement [6, 7, 9, 10]. However, most available evidence stems from heterogeneous cohorts that combine revision and primary procedures, leaving a distinct knowledge gap regarding the performance and specific early failure modes of RHK implants exclusively in non-revision, complex primary settings [6, 7, 9, 10]. These outcomes are particularly encouraging given that using standard implants in these complex cases would carry a high risk of immediate failure or persistent instability [6, 7, 9, 10]. Functional outcomes in this cohort should be interpreted relative to severe baseline deformity and ligamentous loss rather than benchmarked against uncomplicated primary TKA [6, 7, 8]. Thus, RHK prostheses represent a valuable option in complex reconstructive knee surgery, yet further dedicated single-center evaluations in strictly primary cohorts are needed to better define their early complication profile and functional trajectory [6–10]. Evaluating institutional experience with RHK implants in primary TKA is of great practical value to further clarify surgical indications, expected clinical outcomes, and potential failure modes in challenging patient populations [6–10]. The aim of this study was to evaluate short-term implant survivorship, complications, functional outcomes, range of motion (ROM), coronal alignment, and radiographic stability following primary TKA with an RHK implant in patients with complex primary knee pathology.

METHODS

Study design and source data

This retrospective, single-center, observational cohort study evaluated the clinical, functional, and radiographic outcomes of patients who underwent primary TKA using an RHK prosthesis between December 1, 2023, and December 31, 2025. Final clinical and radiographic follow-up data collection was concluded on July 1, 2026, ensuring that all included patients – including the most recently operated cases – had at least six months of potential follow-up time to evaluate early outcomes and survivorship. All patients were tracked continuously from the index surgery, and those experiencing early adverse events or revisions remained within longitudinal institutional follow-up. Data were extracted retrospectively from the institutional electronic health information system, incorporating inpatient medical records, operative protocols, anesthesia records, discharge summaries, and outpatient follow-up notes.

Patient selection criteria

Inclusion criteria were: 1) patients aged > 18 years at index surgery; 2) primary TKA utilizing an RHK prosthesis; 3) surgical indication defined as severe end-stage knee joint degeneration accompanied by gross coronal plane deformity, massive bone defects, and/or severe collateral ligament instability; 4) minimum clinical and radiographic follow-up of six months.

Exclusion criteria were: 1) revision TKA; 2) resection arthroplasty for oncological indications (tumor megaprostheses); 3) active local or systemic infection at index surgery; 4) incomplete medical records lacking primary outcome parameters.

Study variables and clinical evaluation

Collected variables were categorized into three chronological phases: preoperative, intraoperative, and postoperative parameters. Preoperative variables included demographic characteristics, American Society of Anesthesiologists (ASA) physical status classification, and Charlson comorbidity index. Clinical characterization incorporated primary surgical indication, history of prior ipsilateral non-arthroplasty knee surgeries, and type of joint instability (isolated coronal/mediolateral, isolated sagittal/anteroposterior, or global/multiligamentous). Preoperative functional status was evaluated using the OKS, and ROM was measured using a standard goniometer. Full-length weight-bearing standing radiographs were analyzed to measure the preoperative mechanical femorotibial angle (mFTA), with positive values denoting *valgus* deformity and negative values denoting *varus* deformity.

Intraoperative variables recorded included type of anesthesia, application of modular metal augments or autologous bone grafts, stem fixation technique (cementless, cemented, or hybrid), and polyethylene liner thickness (mm). Postoperative variables comprised final follow-up OKS, maximum ROM following rehabilitation, postoperative mFTA, and detailed serial radiographic evaluation.

Surgical technique and perioperative protocol

Surgeries were performed under general or spinal anesthesia after adequate medical optimization. All procedures were carried out by a dedicated team of 10 fellowship-trained orthopedic specialists with extensive expertise in knee arthroplasty. Following standard positioning, an anterior midline skin incision was performed, followed by a medial parapatellar arthrotomy. Upon joint exposure, the preoperative decision for an RHK implant (Rotating Hinge Knee, RHK®, Zimmer Biomet, Warsaw, IN, USA) was confirmed intraoperatively.

Distal femoral and proximal tibial resections were executed using dedicated intramedullary and extramedullary alignment guides, adhering to planned resection angles and depths. Bony defects were reconstructed using modular metal augments or autologous bone grafting according to surgeon preference. Following medullary canal preparation and trial component placement, joint kinematics, gap balance, and hinge stability were verified through a full arc of motion. Final components were implanted using bone cement, obligatorily over resected articular surfaces, and optionally along medullary canal extensions depending on stem fixation protocol. Pulsatile lavage irrigation was performed, and the wound closed in anatomical layers.

Prophylactic first-generation cephalosporins were administered until the first postoperative day. Venous thromboembolism prophylaxis with low-molecular-weight heparin was maintained for five weeks postoperatively. Structured physical therapy commenced on the first postoperative day and was finalized at inpatient rehabilitation centers after hospital discharge.

Radiographic evaluation

Serial anteroposterior and lateral knee radiographs obtained at final follow-up were evaluated by two independent orthopedic surgeons not involved in index operations. Radiographic assessment aimed to detect radiolucent lines > 2 mm according to modern Knee Society zones, component migration, osteolysis, or aseptic loosening. Mechanical alignment correction was determined by comparing preoperative and postoperative mFTA values.

Study outcomes

Primary outcomes were implanting survivorship – defined as time (in months) from index surgery to revision surgery for any cause (involving exchange or removal of any component, including the liner) – and complication rates, categorized as intraoperative, early postoperative (< 90 days), or late postoperative (≥ 90 days).

Secondary outcomes included functional score improvement (mean absolute change in OKS, ΔOKS), gain in ROM, mechanical axis correction (absolute change in mFTA), and incidence of radiographic instability or loosening.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were evaluated for normality using the Shapiro–Wilk test. Normally distributed continuous variables are presented as mean ± standard deviation (m ± SD); non-normally distributed continuous variables are presented as median with interquartile range (IQR). Categorical variables are reported as frequencies and percentages (n, %).

Preoperative and postoperative continuous metrics (OKS, ROM, mFTA) were compared using the paired t-test for parametric data or Wilcoxon signed-rank test for non-parametric data. Time-to-event data were analyzed using the Kaplan–Meier survival analysis method, calculating cumulative survivorship with 95% confidence intervals (CI), setting revision for any cause as the primary endpoint. To identify independent risk factors for revision, variables demonstrating a univariate statistical trend ($p < 0.10$) were entered into a Cox proportional hazards regression model, calculating hazard ratios with 95% CIs. A two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

Demographics and preoperative patient profile

Out of 3921 primary total knee arthroplasties performed at our institution between December 1, 2023, and December 31, 2025, a total of 32 patients underwent primary TKA utilizing an RHK prosthesis. The mean age of the cohort was 67.34 ± 10 years (range: 42–82 years), with a marked

female predominance (25 females *vs*. 7 males; 78.1% *vs*. 21.9%). The median postoperative follow-up was 20.33 months (IQR: 9–24.75 months).

Primary surgical indications comprised primary osteoarthritis (n = 14; 43.8%), secondary osteoarthritis (n = 7; 21.9%), post-traumatic arthritis (n = 7; 21.9%), neuropathic arthropathy (n = 3; 9.4%), and nonunion of the proximal tibia (n = 1; 3.1%). Preoperative ligamentous instability was confirmed in 28 patients (87.5%), most commonly presenting as coronal/mediolateral instability (n = 18; 56.3%) or global multiligamentous instability (n = 9; 28.1%), whereas isolated sagittal instability was noted in a single case (3.1%). Regarding comorbidity indices, the majority of patients belonged to ASA physical status Class II (n = 15; 46.9%) or Class III (n = 14; 43.8%), with a median Charlson comorbidity index of three (IQR: 2–5). Complete demographic and baseline data are detailed in Table 1.

Table 1. Demographic and preoperative patient baseline profile (n = 32)

Variable name	Value/distribution (n = 32)
Age at index surgery (years), mean ± SD (range)	67.34 ± 10 (42–82)
Follow-up duration (months), median (IQR)	20.33 (9–24.75)
Charlson comorbidity index, median (IQR)	3 (2–5)
Prior non-arthroplasty knee surgeries, median (IQR)	0 (0–0) (range: 0–7)
Gender, n (%) • Male (1) • Female (2)	 7 (21.9%) 25 (78.1%)
Surgical side, n (%) • Right (1) • Left (2)	 13 (40.6%) 19 (59.4%)
American Society of Anesthesiologists classification status, n (%) • Class I • Class II • Class III	 3 (9.4%) 15 (46.9%) 14 (43.8%)
Primary surgical indication, n (%) • Primary osteoarthritis (1) • Secondary osteoarthritis (2) • Post-traumatic osteoarthritis (3) • Neuropathic osteoarthritis (4) • Proximal tibial nonunion (5)	 14 (43.8%) 7 (21.9%) 7 (21.9%) 3 (9.4%) 1 (3.1%)
Preoperative knee instability profile, n (%) • No instability (0) • Coronal / mediolateral (1) • Sagittal / anteroposterior (2) • Global / multiligamentous (3)	 4 (12.5%) 18 (56.3%) 1 (3.1%) 9 (28.1%)

Surgical and intraoperative parameters

Spinal anesthesia was administered in 26 patients (81.2%), and a tourniquet was utilized in 22 operations (68.8%). Bone defect reconstruction was required in nine cases (28.1%): modular femoral augments were used in three patients, tibial augments in three patients, and autologous bone grafting for tibial defects in three patients. Bony defect reconstruction was performed using modular metal augments or autologous bone grafting based on defect geometry, intraoperative surgeon judgment, and implant augment availability; specifically, bone grafting was chosen in three tibial defect cases due to augment non-availability in two instances and superior anatomical conformity of the graft to the defect shape in the third.

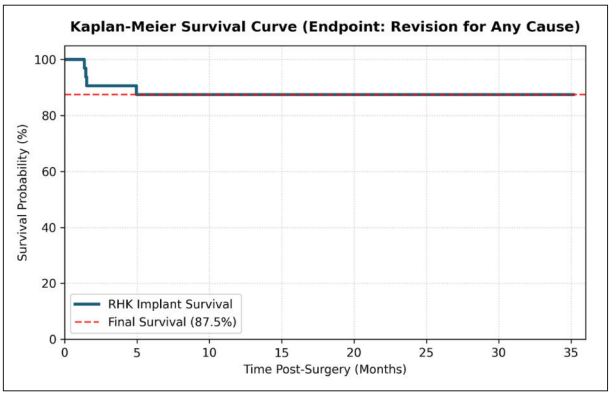


Figure 1. Kaplan–Meier survival curve for rotating hinge knee (RHK) in primary total knee arthroplasty, in months

Cementless stem fixation was predominantly utilized (n = 27; 84.4%). Cemented fixation of both femoral and tibial stems was performed in four cases (12.5%), whereas hybrid fixation (cementless femoral / cemented tibial stem) was used in one patient (3.1%). The most frequently implanted polyethylene liner thickness was 12 mm (n = 25; 78.1%), with an overall liner thickness range between 12 mm and 20 mm.

Implant survivorship (primary outcome)

The primary endpoint was defined as cumulative RHK construct survivorship free from revision surgery for any cause (exchange or removal of any component, including the polyethylene insert). Cumulative 20-month implant survivorship was 87.5% (Figure 1).

All revisions (n = 4) occurred early – within the first five months postoperatively; no late mechanical failures requiring revision were observed during follow-up. Two patients with early PJI underwent "DAIR" protocol (debridement, antibiotics, and implant retention) with polyethylene liner exchange. In one patient who had undergone primary TKA combined with open reduction and double-plate internal fixation for a pre-existing proximal tibial fracture, "DAIR" protocol was performed along with partial hardware removal due to superficial wound dehiscence. In one case of early PJI accompanied by femoral component instability, a two-stage revision was successfully executed, with complete implant removal and temporary placement of an antibiotic-impregnated cement spacer in the first stage.

Complication profile and radiographic status

The overall postoperative complication rate was 34.4% (n = 11). Intraoperative complications occurred in two patients: partial avulsion of the patellar tendon insertion (repaired intraoperatively using bioabsorbable suture anchors) and an iatrogenic femoral shaft fracture (identified on immediate postoperative radiographs and managed

Table 2. Chronological distribution of postoperative complications and treatment strategies

Time window	Observed complication type (n)	Re-operation / treatment strategy
Intraoperative (n = 2)	• Periprosthetic femur fracture (n = 1) • Partial patellar tendon avulsion (n = 1)	• Identified postoperatively; treated nonoperatively with cast immobilization • Repaired intraoperatively with anchors; no secondary revision
Early postoperative (< 90 days) (n = 8)	• Early periprosthetic joint infection (n = 6) • Periprosthetic femur fracture (n = 1) • Peroneal nerve palsy (n = 1)	• **Revision required (n = 4)**: three cases treated via debridement, antibiotics, and implant retention with liner exchange; one case treated via two-stage revision with spacer; two cases successfully treated with IV antibiotics alone • Managed nonoperatively • Complete functional recovery at six months with physical therapy
Late postoperative (≥ 90 days) (n = 1)	• Lateral patellar subluxation (n = 1)	• Managed nonoperatively
Radiographic failures	• Aseptic loosening, osteolysis, or component migration (n = 0)	• **Zero events (0%)** observed across the entire cohort during follow-up

Table 3. Preoperative *vs.* postoperative clinical, functional, and alignment parameters

Outcome parameter	Preoperative status	Postoperative status	Absolute change (δ)	p
Oxford knee score mean ± SD	13.25 ± 5.62	41.09 ± 3.74	**+27.84 ± 7.38**	**< 0.001**
Range of motion median (IQR)	70° (65°–80°)	115° (110°–120°)	**+45°**	**< 0.001**
Mechanical femorotibial angle mean ± SD	13.48° ± 26.36°	6.8° ± 2.86°	Variable	**< 0.001**

conservatively with cast immobilization and restricted weight-bearing).

Early postoperative complications (< 90 days) developed in eight cases, dominated by early PJI (n = 6). Among these, four cases required surgical revision (as detailed above), whereas two cases were successfully eradicated using targeted intravenous antibiotic therapy alone. Early complications also included one post-traumatic distal femoral periprosthetic fracture (managed nonoperatively) and one transient peroneal nerve palsy that achieved complete recovery after six months of physical therapy. Late complications (≥ 90 days) occurred in a single patient as lateral patellar subluxation, which was managed conservatively.

Serial radiographic analysis at final follow-up revealed no progressive radiolucent lines (> 2 mm according to Knee Society criteria), nor any signs of aseptic loosening, osteolysis, or component migration in any case. A chronological summary of complications and surgical management strategies is presented in Table 2.

Functional outcomes and alignment correction

Following operative treatment and complete physical rehabilitation, highly significant statistical improvements were recorded across all functional metrics. The mean OKS rose significantly from a preoperative baseline of 13.25 ± 5.62 to a postoperative mean of 41.09 ± 3.74, representing an absolute functional gain of ΔOKS = +27.84 ± 7.38 points ($p < 0.001$). ROM likewise demonstrated a statistically significant increase, with the median arc expanding from 70° preoperatively to 115° postoperatively ($p < 0.001$).

The RHK system demonstrated exceptional capability in correcting severe coronal alignment deformities. The raw preoperative mean mFTA was 13.48° ± 26.36°, which was corrected to a mean postoperative anatomical alignment of 6.8° ± 2.86° *valgus*. To account for mathematical cancellation of opposing deformities (*varus vs. valgus*), paired t-testing was performed on the absolute deviation from target neutral alignment (6° *valgus*). Absolute coronal plane deformity improved significantly from a preoperative mean of 23.9° to a postoperative mean of 2.36° ($t = 9.42$, $p < 0.001$). Preoperative and postoperative functional scores, ROM, and alignment values are summarized in Table 3.

DISCUSSION

RHK prostheses represent the highest level of implant constraint in knee arthroplasty, where construct stability depends entirely on the mechanical hinge linkage rather than on native joint stabilizers such as collateral or cruciate ligaments. Historically, RHK designs were reserved strictly for complex revision TKAs characterized by massive bone defects and uncorrectable instability. First-generation hinged prostheses suffered from unacceptably high mechanical failure rates, driven by intense stress transmission to the bone-implant-cement interface, which resulted in early aseptic loosening [11]. Second-generation designs introduced a rotating axis that accommodated axial rotation on the tibial plateau, significantly attenuating torsional shear stresses on the host bone. Third-generation systems further incorporated modular stem options, anatomical patellofemoral trochlear kinematics, highly conformal polyethylene inserts, and modular metal augments for joint line restoration and bone defect reconstruction [12].

These design refinements have broadened the clinical application of RHK implants to encompass complex primary TKAs. Neuhaus and Maier [7] recently confirmed that while RHK constructs were historically limited to salvage procedures, contemporary design iterations provide safe and predictable primary joint reconstruction when native collateral ligaments are non-functional. Our cohort achieved favorable 20-month cumulative RHK survivorship of 87.5% in complex primary knees. Furthermore, patients demonstrated a dramatic functional improvement,

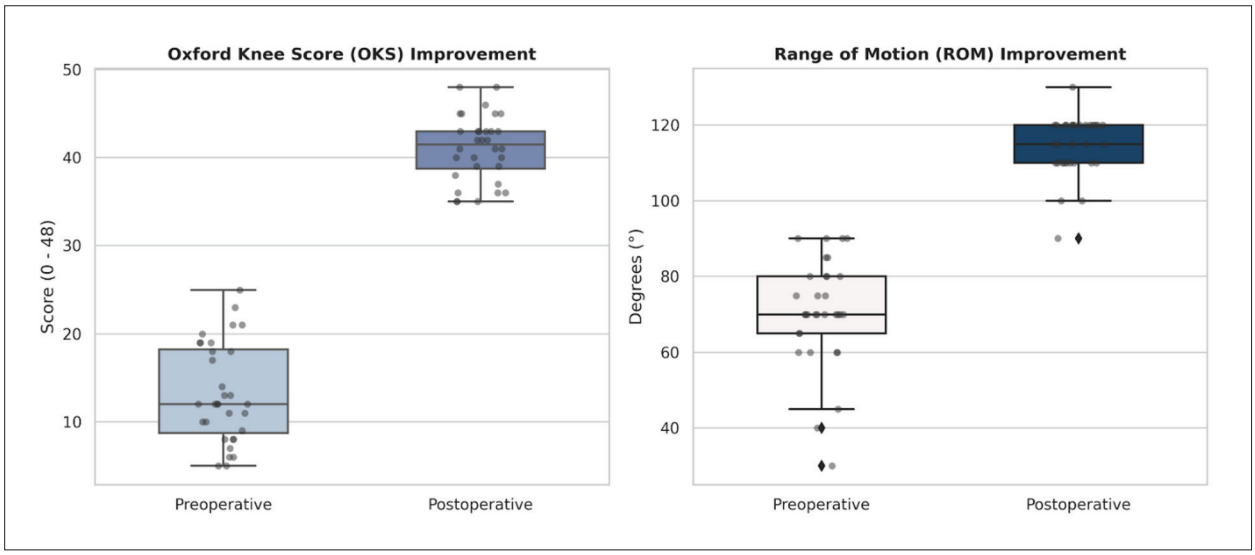


Figure 2. Functional Oxford knee score (OKS) and range of motion (ROM) improvement after rotating hinge knee primary total knee arthroplasty

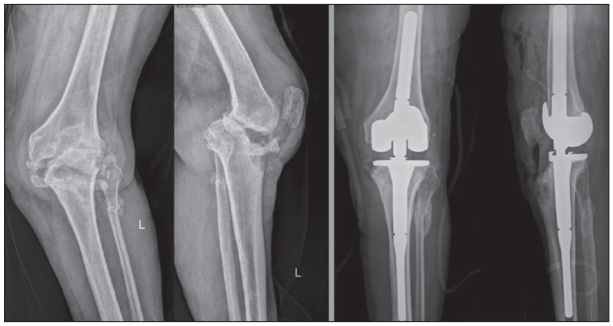


Figure 3. Preoperative X-ray of 67-year-old female patient with severe posttraumatic osteoarthritis, *valgus* deformity and antero-posterior instability (left); postoperative X-ray after primary total knee arthroplasty with rotating hinge knee (right)

evidenced by a mean ΔOKS gain of +27.84 points and complete elimination of preoperative joint laxity. These findings closely mirror the landmark findings by Puri et al. [6] from the Hospital for Special Surgery, who reported a 95% five-year reoperation-free survivorship and 100% survivorship free from aseptic loosening in 54 complex primary RHK cases. These collective findings underscore that RHK prostheses are no longer limited to revision salvage, but represent a crucial primary option when native joint stabilizers are irreversibly lost [6, 7].

Reported mid- and long-term survivorship rates for RHK implants in primary settings vary considerably across the literature depending on follow-up duration and the specific definition of endpoints (all-cause revision *vs*. re-operation) [9, 13–16]. In a comprehensive meta-analysis of 6554 primary knees, Abdel Khalik et al. [9] demonstrated a five-year revision-free survival rate of 93.8% and a 10-year survival rate of 91.5%, establishing robust global benchmarks for primary RHK implants. Similarly, von Hintze et al. [14] documented a 10-year survivorship of 91.6% in a dedicated primary cohort of 106 knees, while Schaible et al. [13] reported a 100% implant survival rate at a mean follow-up of 32 months. Conversely, some older series reported guarded outcomes, with five-year survival dropping as low as 52% [15]. In the longest single-center series (13.5 years mean follow-up), Kendoff et al. [16] demonstrated that younger age (< 60 years) and preoperative *valgus* deformity significantly increased revision risk (13.5-year survival of 96% in severe *varus vs*. 64% in *valgus* knees). In this context, our observed revision-free survivorship of 87.5% reflects an early- to mid-term observation window (median follow-up of 20.3 months); although direct comparison with five- and 10-year register benchmarks is limited by this time horizon discrepancy, our early trajectory is encouraging, and longer longitudinal follow-up is warranted to confirm whether these results remain comparable over the long term.

A paramount finding in our study is the complete absence of aseptic component loosening, osteolysis, or component migration (0%) throughout the follow-up window. While this is partially attributable to the short follow-up duration, it highlights the mechanical efficacy of modern RHK mechanisms combined with long stem extensions in dissipating torsional shear loads at the bone-implant interface [6]. Cottino et al. [17] corroborated this mechanical durability, demonstrating an exceptionally low 10-year cumulative revision rate for aseptic loosening of just 4.5% across 408 RHK knees. Furthermore, Moussa et al. [18] confirmed that intramedullary stem extensions in constrained primary TKA significantly enhance construct rigidity and eliminate early mechanical failure compared to non-stemmed constrained designs. Our experience aligns with Kowalczewski et al. [19], who achieved 100% 10-year survival free from mechanical loosening using hybrid press-fit long stems, and Spranz et al. [8], who proved in a dedicated cohort that adding metaphyseal modular interfaces to RHK stems does not compromise primary fixation stability ($p = 0.072$).

Etiological and chronological analysis of our failures ($n = 4$ revisions) demonstrates that implant loss was driven exclusively by early PJI and soft-tissue healing problems rather than mechanical loosening or material fatigue (all

revisions occurred within five months postoperatively). Severe preoperative deformities, prolonged operative times, and compromised host health (high ASA physical status and comorbidity indices) render this patient population highly vulnerable to infectious complications [11, 20]. In their meta-analysis, Abdel Khalik et al. [9] showed an overall rate of complications requiring revision of 7.4%, with the most common causes being infection (2.6%), aseptic loosening (1.2%), and periprosthetic fracture (1.1%). PJI as the dominant early failure mode is further corroborated by Bruce et al. [12], who evaluated 53 RHK implants at a mean 7.6-year follow-up and confirmed that soft-tissue complications and PJI represent the primary causes of early secondary intervention, as well as Rouquette et al. [20], who emphasized that septic risks are substantially heightened in patients with prior non-arthroplasty surgeries and compromised soft-tissue envelopes. Furthermore, given the extreme deformities and extensive posterior capsular releases frequently required in complex primary RHK TKA, meticulous intraoperative dissection is mandatory to avoid catastrophic neurovascular complications, such as iatrogenic popliteal artery injury [21].

Functional recovery in our cohort was remarkable, characterized by a dramatic increase in mean OKS (from 13.25 ± 5.62 preoperatively to 41.09 ± 3.74 postoperatively) and expansion of median ROM to 115°. This high functional ceiling is facilitated by the primary TKA setting, where periarticular soft tissues are well preserved, unlike in revision TKA [11, 20, 22]. In a prospective matched-pair analysis comparing primary RHK directly against standard primary TKA, Schaible et al. [13] demonstrated that primary RHK achieved statistically superior postoperative ROM (mean 126°) compared to matched standard TKA (117°, $p = 0.012$), alongside complete elimination of mediolateral laxity (which was present in 26% of standard primary TKAs). Our mean postoperative ROM of 115° matches the findings of Hecker et al. [11], while our postoperative OKS score of 41.09 aligns with contemporary patient-reported outcome expectations highlighted in recent meta-analyses by Park et al. [5] and matched-pair satisfaction studies by Vogel et al. [2].

The geometric restoration of limb alignment (reducing absolute coronal deviation from 23.9° preoperatively to 2.36° postoperatively) highlights the biomechanical superiority of the RHK construct. Hecker et al. [11] emphasized that severe uncorrectable coronal deformities constitute a primary indication for RHK implantation. Our radiographic results align with Kendoff et al. [16], confirming that primary RHK provides exceptional alignment correction in severe *varus* deformities (96% long-term survival), while emphasizing that younger *valgus* patients require rigorous surgical precision and vigilant monitoring. Furthermore, as demonstrated by DeFrance et al. [3] and Singh et al. [1], restoring joint stability and overall mechanical alignment in complex primary knees directly correlates with achieving modern patient satisfaction rates above 85–90%.

Long-term surveillance remains essential given the systemic frailty of this cohort. In their 15-year landmark study, Yang et al. [23] noted that despite an 87% implant survival rate, 100% of primary RHK patients eventually required ambulatory gait aids (canes or walkers), with a cumulative late PJI rate of 14%. This indicates that reduced patient mobility in long-term follow-up is frequently driven by systemic, neurological, or medical comorbidity progression rather than mechanical TKA failure, which must be clearly communicated during preoperative patient counseling [6, 23, 24].

This study has several limitations. First, its retrospective single-center design carries inherent risk of selection bias. Second, follow-up duration (median 20.3 months) is optimal for detecting early soft-tissue and infectious failures, but insufficient for evaluating long-term polyethylene wear or late aseptic loosening [11, 17].

CONCLUSION

Primary TKA using a contemporary RHK prosthesis is a highly effective, reliable, and biomechanically sound procedure for patients presenting with severe coronal plane deformities, gross ligamentous instability, and major bone defects. The procedure yields dramatic, statistically significant functional recovery, substantial pain relief, and accurate mechanical axis restoration. With an 87.5% cumulative implant survivorship at median 20.3 months and a 0% rate of early mechanical loosening or component migration, modern RHK designs demonstrate excellent early structural durability. However, because early failures are driven exclusively by PJI and wound healing complications, long-term success relies on strict preoperative patient selection, meticulous soft-tissue handling, and aggressive infection prevention measures.

Ethical approval and consent to participate: The subjects' written consent was obtained, according to the Declaration of Helsinki. The study has been approved by the Institutional Ethics Committee (approval number I-226/24) and conducted in accordance with legal standards and STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for observational studies.

Conflict of interest: None declared.

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Клинички, функционални и радиографски исходи примарне тоталне артропластике колена уз примену импланта са ротационом шарком – ретроспективна кохортна студија

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САЖЕТАК

Увод/Циљ Циљ рада био је процена клиничких, функционалних и радиографских исхода, као и преживљавања имплантата након примарне тоталне артропластике колена уз примену протезе са ротационом шарком код комплексних примарних случајева.

Методе Ова ретроспективна, једноцентрична опсервациона кохортна студија обухватила је 32 болесника (32 колена) оперисана у периоду од 1. децембра 2023. до 31. децембра 2025. године. Примарни исходи били су кумулативна стопа преживљавања имплантата (Каплан–Мајерова анализа са ревизијом из било ког разлога као крајњом тачком) и стопа компликација. Секундарни исходи обухватали су функционални статус процењен помоћу Оксфордског упитника за процену функције колена (*OKS*), обим покрета, корекцију механичког феморотибијалног угла и радиографску стабилност.

Резултати Медијана периода праћења износила је 20,3 месеца. Индикације за уградњу биле су примарни (43,8%) и секундарни остеоартритис (21,9%), посттрауматски артритис (21,9%), неуропатска артропатија (9,4%) и псеудоартроза проксималне тибије (3,1%). Преоперативна лигаментарна нестабилност верификована је код 87,5% болесника. Кумулативна 20-месечна стопа преживљавања имплантата износила је 87,5% (све четири ревизије извршене су у првих пет месеци након операције услед ране перипротетске инфекције и компликација ране; стопа асептичног разлабављења износила је 0%). Укупна стопа компликација износила је 34,4%. Постоперативно, средња вредност *OKS* порасла је са 13,25 ± 5,62 на 41,09 ± 3,74 (Δ*OKS* = +27,84, $p < 0,001$), медијана обима покрета са 70° на 115° ($p < 0,001$), а апсолутни коронални деформитет (одступање од неутралне биомеханичке осовине) смањио се са 23,9° на 2,36° ($p < 0,001$).

Закључак Примарна тотална артропластика колена уз примену протезе са ротационом шарком обезбеђује одличан функционални опоравак, високу рану механичку стабилност и поуздану корекцију деформитета код комплексних примарних стања, при чему су ригорозна контрола инфекције и пажљиво поступање са меким ткивима од кључног значаја.

Кључне речи: тотална артропластика колена; протеза са ротационом шарком; примарна артропластика; анализа преживљавања; функционални исход

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

ZIP6 and ZIP14 zinc transporter expression in normozoospermic and asthenozoospermic human spermatozoa – relationship to nuclear chromatin accessibility

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SUMMARY

Introduction/Objective Male infertility accounts for approximately 40% of infertility cases, with asthenozoospermia affecting nearly one in five infertile men. Beyond conventional semen parameters, nuclear DNA organization and chromatin accessibility provide deeper insight into sperm quality. Zinc transporters ZIP6 and ZIP14 have been implicated in chromatin stabilization and zinc homeostasis, potentially essential to sperm maturation and fertilization competence.

Methods Semen samples from normozoospermic and asthenozoospermic men undergoing intrauterine insemination were purified by density gradient. ZIP6 and ZIP14 were detected by immunofluorescence, nuclear DNA visualized by using Sytox™ Orange (Thermo Fisher Scientific, Waltham, MA, USA). Expression levels, nuclear area, and staining intensity were quantified in ImageJ (National Institutes of Health), and analyzed using Kruskal–Wallis test with Dunn's post-hoc correction ($p \leq 0.05$).

Results ZIP6 immunopositivity was significantly reduced in asthenozoospermic compared to normozoospermic samples, while ZIP14 expression remained stable. Nuclear area and overall Sytox™ Orange (Thermo Fisher Scientific) intensity did not differ significantly between transporter-positive and -negative sperm, though ZIP6-positive asthenozoospermic cells showed a modest reduction in nuclear surface area. Normozoospermic ZIP14-negative spermatozoa displayed lower Sytox™ Orange (Thermo Fisher Scientific) fluorescence than ZIP6-negative cells, suggesting focal differences in chromatin accessibility between transporter-defined subpopulations.

Conclusion ZIP6 expression reflects sperm maturation and functional competence, while ZIP14 remains constitutively stable. Both transporters may serve as molecular markers for selecting high-quality sperm in assisted reproduction, particularly in populations exposed to occupational stressors that compromise DNA integrity.

Keywords: ZIP6; ZIP14; spermatozoa; chromatin accessibility; asthenozoospermia; normozoospermia

INTRODUCTION

Male infertility contributes to approximately 40% of infertility cases [1], and while routine semen analysis focuses on concentration, motility, and morphology, increasing attention has been directed toward nuclear integrity as an additional dimension of sperm quality [2].

Spermatozoa represent a highly heterogeneous cell population, both morphologically and molecularly, even within normozoospermic samples. This heterogeneity includes differences in chromatin compaction, oxidative susceptibility, and protein composition, arising during spermatogenesis and epididymal maturation and reflecting variations in nuclear stability, capacitation potential, and protein expression profiles, including zinc transporters [3, 4]. Proper chromatin packaging is essential for sperm functional competence, as the paternal genome must remain transcriptionally silent and structurally protected until fertilization.

Chromatin remodeling and DNA protection mechanisms

During spermatogenesis, histones are sequentially replaced by transition proteins and protamines, resulting in highly compacted toroidal chromatin stabilized by disulfide bonds. This organization renders the sperm genome transcriptionally inactive and provides strong protection against oxidation, elevated temperatures in the female reproductive tract, and other external stressors [5, 6, 7]. However, the degree of chromatin compaction is not uniform across all spermatozoa, and incomplete protamination or structural irregularities may compromise nuclear integrity and DNA stability. Such imperfections, arising from defective histone-to-protamine transition, impaired recombination or repair, or abortive apoptosis, result in a baseline level of DNA damage detectable in testicular, epididymal, and ejaculated spermatozoa. Importantly, inadequate chromatin condensation may leave portions of the genome in a transcriptionally accessible state, which is at odds with the biological

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requirement for transcriptional silencing of the paternal genome prior to fertilization [3, 5].

Factors contributing to sperm DNA damage

Beyond intrinsic spermatogenic imperfections, sperm DNA damage has been associated with multiple lifestyle, environmental, and health-related factors, including smoking, environmental pollutants, and other conditions that promote oxidative stress [8]. Although damaged germ cells are typically eliminated via apoptosis, some may escape this process and still participate in fertilization, with potential consequences for embryo development and pregnancy outcomes [6, 9, 10].

Zinc (Zn^{2+}) in male reproductive physiology

Zinc plays a crucial role in chromatin stabilization during spermatogenesis. It contributes to DNA condensation by stabilizing protamine–DNA interactions and supporting the formation of disulfide bonds that reinforce nuclear structure [11, 12]. Through these mechanisms, zinc plays an important role in maintaining chromatin stability and protecting sperm DNA from fragmentation [10–13]. Beyond its structural role, zinc may also function as an additional safeguard upon ejaculation, when exogenous Zn^{2+} is incorporated into the sperm nucleus and helps stabilize chromatin, inhibit premature metabolic activity, preserve membrane integrity, and exert antioxidant effects [11]. In this way, zinc contributes both to DNA integrity and to the acrosome reaction, with membrane-associated transporters such as ZIP6 and ZIP14 influencing intracellular zinc availability [12, 14, 15]. This raises the possibility that differences in zinc transporter expression among individual spermatozoa could underlie part of the functional heterogeneity observed within the ejaculate.

Zinc transporters as determinants of sperm subpopulations

Zinc homeostasis in spermatozoa is regulated through the coordinated activity of ZIP importers, which increase cytosolic zinc, and ZnT exporters, which reduce it. In our previous study, we characterized the localization and differential expression of ZIP6 and ZIP14 in normozoospermic and asthenozoospermic spermatozoa, demonstrating the existence of distinct ZIP-positive and ZIP-negative subpopulations within the ejaculate [16]. These findings suggest that individual sperm cells differ in their capacity for zinc uptake, providing a molecular basis for functional diversity. Whether this variability in zinc transporter expression is associated with differences in nuclear chromatin organization and DNA integrity, however, remains to be established.

Although zinc transporters of the ZIP family define distinct subpopulations of ZIP-positive and ZIP-negative spermatozoa within the ejaculate, it remains unknown whether these subpopulations differ in nuclear chromatin organization or DNA integrity. Therefore, the aim of this study was to examine the association between ZIP6 and ZIP14 expression and nuclear characteristics of human spermatozoa, specifically, nuclear surface area and chromatin accessibility as assessed by Sytox™ Orange (Thermo Fisher Scientific, Waltham, MA, USA) fluorescence intensity in both normozoospermic and asthenozoospermic samples.

METHODS

Sample collection

Semen samples were obtained from male patients undergoing intrauterine insemination at the University Clinical Center of Serbia, Višegradska Clinic for Gynecology and Obstetrics, Department for Medically Assisted Reproduction, Belgrade, Serbia, and written informed consent was obtained from all participants prior to sample collection. The sperm samples used in this study were drawn from the same cohort previously analyzed for compartmental and subcompartmental ZIP6 and ZIP14 localization [16]; the present analysis extends that work by examining the relationship between transporter expression and nuclear characteristics.

Sample preparation and classification

A total of 50 individual semen samples were collected in sterile containers following 3–5 days of sexual abstinence and allowed to liquefy at room temperature. Semen quality was assessed according to 2010 World Health Organization laboratory guidelines [17], and samples were classified as normozoospermic or asthenozoospermic. Men with known risk factors for male infertility or relevant medical history were excluded. For in-depth cellular analysis, four samples were selected, two normozoospermic and two asthenozoospermic, from donors aged 31–36 years, without children, and with a mean body mass index of 26.83.

Spermatozoa were purified using a two-layer density gradient to remove leukocytes, non-spermatic cells, and immotile spermatozoa. SpermGrad (Vitrolife®, Göteborg, Sweden) solutions at 90% and 45% were layered and centrifuged at 300 g for 20 minutes. The resulting pellet was washed twice in 5 mL of 0.9% NaCl at 300 g for 15 minutes each, then resuspended in 0.5 mL of 0.9% NaCl [16]. Sperm smears were prepared from the final suspension for immunofluorescence analysis.

Immunofluorescence

Smears were fixed in methanol for 15 minutes, air-dried, then post-fixed and permeabilized in acetone for five minutes, followed by incubation in 0.3% Triton X-100 in Tris-buffered saline (TBS) for 10 minutes. Non-specific binding was blocked for 30 minutes using a solution of 0.1%

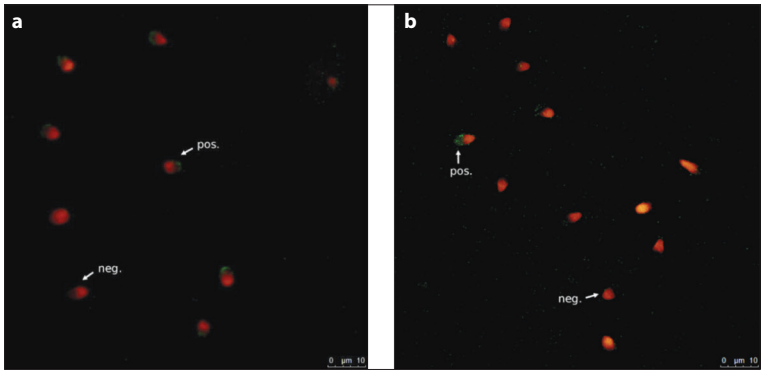


Figure 1. Sytox™ Orange (Thermo Fisher Scientific, Waltham, MA, USA) reaction intensity in the ZIP6 positive (+, pos.) and negative (-, neg.) reactions in normozoospermic (a) and asthenozoospermic (b) semen samples using confocal laser scanning microscopy, scale bar = 10 µm

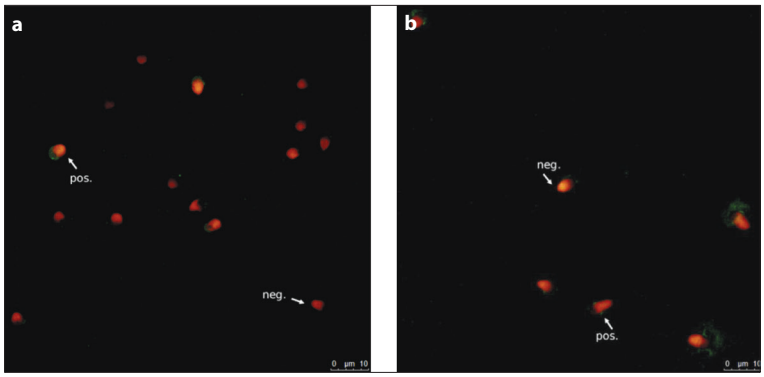


Figure 2. Sytox™ Orange (Thermo Fisher Scientific, Waltham, MA, USA) reaction intensity in the ZIP14 positive (+, pos.) and negative (-, neg.) reactions in normozoospermic (a) and asthenozoospermic (b) semen samples using confocal laser scanning microscopy, scale bar = 10 µm

bovine serum albumin, 3% normal goat serum, 0.2% Triton, and 0.05% Tween 20 in TBS. Smears were then incubated overnight at 4°C with primary antibodies against ZIP6 (1:50, PA5-21071, Invitrogen, Thermo Fisher Scientific) and ZIP14 (1:50, PA5-21077, Invitrogen, Thermo Fisher Scientific) diluted in blocking solution. After three washes in TBS with Tween 20, smears were incubated for one hour at room temperature with Alexa Fluor 488-conjugated secondary antibody (1:400, A-11029, Invitrogen, Thermo Fisher Scientific). Nuclei were counterstained with Sytox™ Orange (Thermo Fisher Scientific) (1:1000, S11368, Life Technologies, Thermo Fisher Scientific) for five minutes, after which slides were rinsed in TBS and mounted in Mowiol (Sigma-Aldrich, St. Louis, MO, USA) [18]. Specificity was confirmed by parallel negative controls in which primary antibodies were omitted.

Images were acquired using a Leica TCS SP5 II confocal microscope (Leica Microsystems, Wetzlar, Germany) operated in sequential mode to prevent channel crosstalk. ZIP immunofluorescence was excited at 488 nm (argon laser) and Sytox™ Orange (Thermo Fisher Scientific) at 543 nm (helium–neon laser). Signals were captured in the green channel (ZIP) and the orange-red channel [Sytox™ Orange (Thermo Fisher Scientific)].

Quantification of immunopositivity

Immunofluorescence images were analyzed using ImageJ (National Institutes of Health) version 1.52p. Spermatozoa displaying any level of ZIP6 or ZIP14 immunoreactivity were classified as ZIP-positive, irrespective of signal intensity or subcellular distribution, while cells showing no detectable signal were classified as ZIP-negative. For each sample, nuclear surface area and Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity were measured in a minimum of 100 spermatozoa per group.

Statistics

Data was analyzed using GraphPad Prism 8 (GraphPad Software, Boston, MA, USA), version 8.4.3. Differences in nuclear area and Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity between ZIP-positive and ZIP-negative spermatozoa were assessed using the Kruskal–Wallis test with Dunn's post-hoc correction for multiple comparisons. Statistical significance was set at $p \leq 0.05$.

RESULTS

As previously reported, ZIP6 immunopositivity was significantly higher in normozoospermic than in asthenozoospermic spermatozoa, while ZIP14 immunopositivity did not differ significantly between groups [16]. Building on those findings, the present study examined whether this differential transporter expression is associated with differences in nuclear characteristics, specifically, sperm head nuclear surface area and Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity as a measure of chromatin accessibility, comparing ZIP-positive and ZIP-negative spermatozoa within both normozoospermic and asthenozoospermic samples (Figures 1 and 2).

Sperm head nuclear surface area

Sperm head nuclear surface area did not differ significantly between ZIP-positive and ZIP-negative spermatozoa in normozoospermic samples for either ZIP6 or ZIP14 (Figure 3a). In asthenozoospermic samples, ZIP6-positive spermatozoa had a slightly but significantly smaller nuclear surface area compared to ZIP6-negative cells, while no such difference was observed for ZIP14 (Figure 3b). Overall, nuclear surface area remained within a comparable range across all groups, suggesting that sperm head morphology is largely consistent regardless of ZIP transporter expression status.

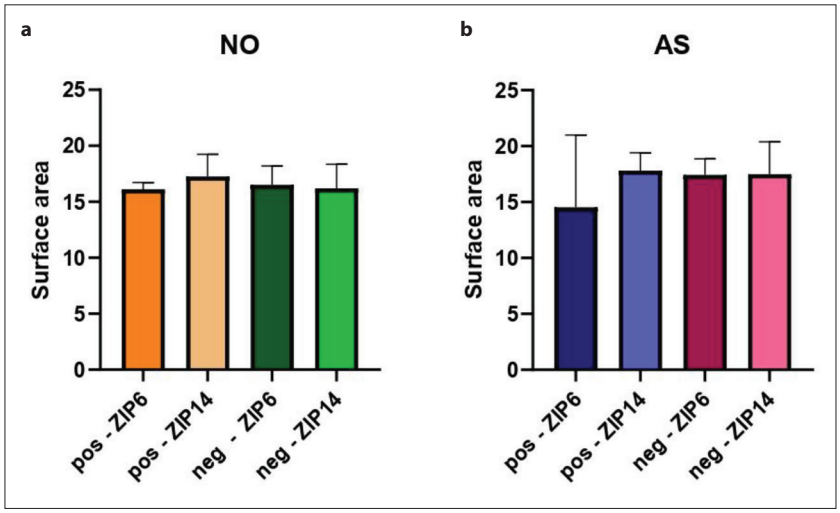


Figure 3. Sperm head nuclear surface area; sample types: a.) NO (normozoospermia); b.) AS (asthenozoospermia); pos-ZIP6, pos-ZIP14 – positive reactions for ZIP; neg-ZIP6, neg-ZIP14 – negative reactions for ZIP

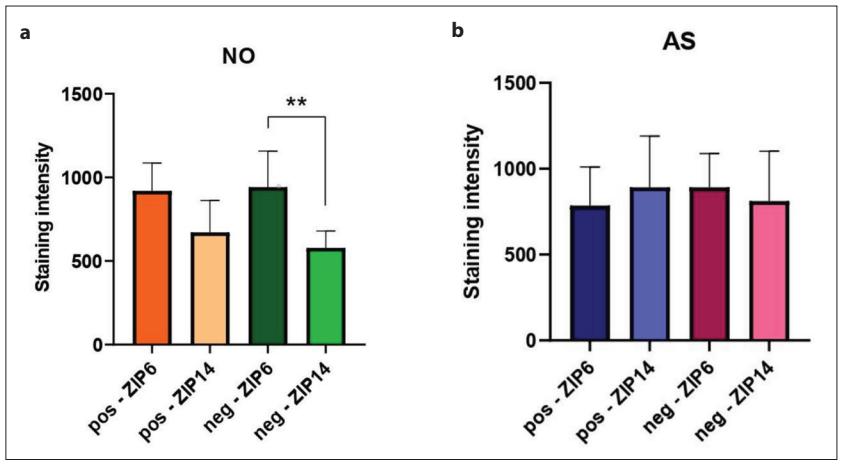


Figure 4. DNA staining intensity with Sytox™ Orange (Thermo Fisher Scientific, Waltham, MA, USA): a.) NO (normozoospermia); b.) AS (asthenozoospermia); pos-ZIP6, pos-ZIP14 – positive reactions for ZIP; neg-ZIP6, neg-ZIP14 – negative reactions for ZIP;
**$p \leq 0.01$

Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity

In normozoospermic samples, Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity did not differ significantly between ZIP6-positive and ZIP14-positive spermatozoa, nor between ZIP6-positive and ZIP6-negative cells (Figure 2a). However, a statistically significant difference was observed between ZIP6-negative and ZIP14-negative spermatozoa, with ZIP14-negative cells displaying lower fluorescence intensity than ZIP6-negative cells ($p \leq 0.01$; Figure 4a). In asthenozoospermic samples, no statistically significant differences in Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity were observed between ZIP-positive and ZIP-negative spermatozoa for either ZIP6 or ZIP14 (Figure 4b).

DISCUSSION

The present study examined whether ZIP6 and ZIP14 zinc transporter expressions are associated with differences in nuclear characteristics among human spermatozoa, using Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity and nuclear surface area as indicators of chromatin accessibility and nuclear morphology. The analysis was performed in ZIP-positive and ZIP-negative subpopulations within normozoospermic and asthenozoospermic samples, building on previously reported compartmental and subcompartmental localization data for these transporters [16].

Consistent with earlier observations, ZIP14 immunoreactivity was relatively stable between normozoospermic and asthenozoospermic groups, whereas ZIP6 expression was reduced in asthenozoospermia [16]. This differential pattern suggests that ZIP6 and ZIP14 reflect distinct aspects of sperm cellular biology rather than equivalent functional roles. The reduction in ZIP6 immunopositivity in asthenozoospermic spermatozoa supports the notion that ZIP6 expression may possibly be linked to sperm maturation status and functional competence, consistent with the recognized role of zinc transporter dynamics in spermatogenesis and maintenance of sperm cellular homeostasis [12, 19]. Whether this reduction reflects impaired zinc uptake capacity, altered transporter trafficking, or simply a marker of a less mature sperm subpopulation remains to be determined. By contrast, the relative stability of ZIP14 across groups suggests that its expression may be maintained independently of motility status, possibly reflecting a more constitutive role in zinc homeostasis.

The central question of this study was whether transporter-defined sperm subpopulations differ in nuclear characteristics. No major differences in sperm head nuclear surface area or Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity were observed between ZIP-positive and ZIP-negative spermatozoa in either group, indicating preserved global nuclear morphology and overall chromatin organization regardless of ZIP expression status. As Sytox™ Orange (Thermo Fisher Scientific) fluorescence reflects DNA accessibility rather than DNA fragmentation, these findings suggest comparable bulk chromatin compaction while not excluding subtle or localized DNA damage detectable only by more sensitive assays [20]. Importantly, the preservation of global nuclear features does not preclude functional heterogeneity. Differential immunoreactivity of ZIP6 and ZIP14 suggests that these transporters may represent distinct aspects of sperm cellular

diversity rather than equivalent functional roles [4]. The immunofluorescence pattern suggests that ZIP6 may be associated with sperm functional competence, supported by its reduced presence in asthenozoospermia and previous links between zinc transporter expression and sperm maturation [12, 15, 19]. Differences between ZIP6 and ZIP14 may reflect transporter-specific regulation during spermatogenesis or define sperm subpopulations with altered chromatin remodeling dynamics despite preserved global nuclear structure.

Beyond its fundamental role in zinc homeostasis, intracellular zinc availability has been increasingly associated with sperm chromatin organization and nuclear heterogeneity [12, 13]. During spermatogenesis, chromatin condensation and the histone-to-protamine transition are tightly regulated processes influenced by histone acetylation and epigenetic remodeling [5, 21]. Recent studies have shown that histone retention, protamine replacement, and post-translational histone modifications contribute to the establishment of localized regions of accessible chromatin within the highly condensed sperm nucleus [3, 22], supporting the concept that spermatozoa within a single ejaculate represent structurally heterogeneous subpopulations which may affect future offspring [23].

Consistent with this concept of nuclear heterogeneity, one finding warrants specific attention. In normozoospermic samples, a statistically significant difference in Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity was observed between ZIP6-negative and ZIP14-negative spermatozoa, with ZIP14-negative cells displaying lower intensity. This may suggest that, among spermatozoa lacking transporter immunoreactivity, the absence of ZIP14 may be associated with reduced DNA accessibility, potentially reflecting differences in chromatin organization compared to cells lacking ZIP6 [22, 23]. This distinction may possibly indicate distinct regulatory roles of the two transporters during spermatogenesis [15, 16]. Zinc is known to contribute to chromatin stabilization through protamine–DNA interactions and disulfide bond formation [11, 14], raising the possibility that ZIP14-dependent zinc availability could influence local chromatin condensation in ways not captured by ZIP6 expression alone. Although this finding is subtle and based on a limited sample, it highlights a biologically meaningful observation that warrants a follow-up with larger cohorts and complementary chromatin assays.

The sperm nuclear surface area findings further support the overall picture of structural consistency across subpopulations. The slightly smaller nuclear area observed in ZIP6-positive asthenozoospermic spermatozoa may reflect a subpopulation with subtly altered chromatin compaction or incomplete nuclear maturation [3, 5, 22], though this observation must be interpreted cautiously given the known sensitivity of nuclear area measurements to fixation and staining conditions [24], and requires confirmation in larger, independent cohorts. This differential pattern suggests that ZIP6 and ZIP14 head dimensions in human spermatozoa are generally tightly constrained by the final stages of spermiogenesis, and it is plausible that major morphological divergence between ZIP-positive and ZIP-negative cells would be unlikely in purified, motility-selected samples [5, 25].

It is important to note that Sytox™ Orange (Thermo Fisher Scientific) intensity, while informative as an indicator of chromatin accessibility, is not equivalent to established measures of DNA integrity such as the DNA fragmentation index obtained by sperm chromatin structure assay (SCSA), the Comet assay, or terminal deoxynucleotidyl transferase dUTP nick end labeling staining [9, 20, 26]. The interpretation of intensity differences reflecting chromatin organization rather than DNA damage is therefore appropriate, but limits the conclusions that can be drawn regarding fertilization competence. Future studies should combine zinc transporter immunoprofiling with validated DNA fragmentation assays in larger, well-characterized cohorts to determine whether ZIP6 or ZIP14 expression predicts sperm DNA integrity in a clinically meaningful sense [27].

Taken together, the present findings indicate that ZIP6 and ZIP14 define distinct sperm subpopulations that do not differ substantially in global nuclear morphology or bulk chromatin staining. The heterogeneity in Sytox™ Orange (Thermo Fisher Scientific) intensity observed among transporter-negative cells in normozoospermia suggests that subtle differences in chromatin organization may exist between ZIP6- and ZIP14-dependent zinc handling pathways, a distinction that Sytox™ Orange (Thermo Fisher Scientific) alone cannot fully resolve. Future research should, therefore, extend to validated assays of sperm DNA fragmentation and chromatin integrity to better elucidate the fertilization potential of these subpopulations and their impact on male reproductive health.

CONCLUSION

This study demonstrates that ZIP6 and ZIP14 identify molecularly distinct subpopulations of human spermatozoa in both normozoospermic and asthenozoospermic samples. The reduced ZIP6 immunopositivity observed in asthenozoospermia, in contrast to the stable expression of ZIP14, suggests a possible association between ZIP6 and sperm maturation status and functional competence.

The absence of pronounced differences in sperm head nuclear surface area and Sytox™ Orange (Thermo Fisher Scientific) fluorescence intensity between ZIP-positive and ZIP-negative spermatozoa indicates that transporter-defined subpopulations do not differ substantially in global nuclear morphology or bulk chromatin organization. The subtle intensity differences observed among transporter-negative cells in normozoospermic samples may reflect focal variations in chromatin accessibility rather than generalized DNA fragmentation, consistent with the known structural heterogeneity of ejaculated sperm populations.

Collectively, these findings suggest that ZIP6 and ZIP14 represent potential molecular markers of sperm heterogeneity whose relevance extends beyond zinc homeostasis to broader questions of sperm nuclear quality. The present results indicate that transporter-defined heterogeneity is

primarily molecular rather than associated with major nuclear structural alterations, highlighting that subtle molecular differences may exist despite preserved global morphology. Given the potential relevance of molecular differences among individual spermatozoa to fertilization potential, future studies integrating zinc transporter immunoprofiling with validated DNA fragmentation assays, such as SCSA, terminal deoxynucleotidyl transferase dUTP nick end labeling, or the Comet assay, in larger and more diverse cohorts, including those exposed to environmental or occupational stressors, may help determine whether differential ZIP expression is associated with DNA damage and ultimately influences fertilization potential and assisted reproductive outcomes.

Ethics: The study was approved by the Ethics Committee of the University Clinical Center of Serbia, Belgrade, Serbia (No.: 1730/12), and written informed consent was obtained from all participants prior to sample collection.

Conflict of interest: None declared.

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Експресија транспортера цинка *ZIP6* и *ZIP14* у хуманим сперматозоидима из нормозооспермичних и астенозооспермичних узорака – повезаност са приступачношћу нуклеарног хроматина

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САЖЕТАК

Увод/Циљ Мушки инфертилитет чини приближно 40% свих случајева неплодности, при чему астенозооспермија погађа скоро сваког петог инфертилног мушкарца. Поред конвенционалних параметара анализе семена, организација нуклеарне ДНК и приступачност хроматина пружају дубљи увид у квалитет сперматозоида. Транспортери цинка *ZIP6* и *ZIP14* доводе се у везу са стабилизацијом хроматина и хомеостазом цинка, процесима који могу бити од суштинског значаја за сазревање сперматозоида и њихову фертилизациону способност.

Методе Узорци семена нормозооспермичних и астенозооспермичних мушкараца укључених у програм интраутерине инсеминације пречишћени су путем градијента густине. *ZIP6* и *ZIP14* детектовани су имунофлуоресценцијом, док је нуклеарна ДНК визуализована применом боје *Sytox™ Orange (Thermo Fisher Scientific,* Валтам, МА, САД). Ниво експресије, површина нуклеуса и интензитет бојења квантификовани су у програму *ImageJ* (Национални здравствени институт), а разлике између група анализиране су Крускал–Валисовим тестом уз Данову *post hoc* корекцију ($p \leq 0,05$).

Резултати Имунопозитивност *ZIP6* била је значајно смањена у астенозооспермичним у односу на нормозооспермичне узорке, док је експресија *ZIP14* остала стабилна. Површина једра и укупни интензитет флуоресценције боје *Sytox™ Orange (Thermo Fisher Scientific)* нису се значајно разликовали између сперматозоида позитивних и негативних на транспортере, иако су *ZIP6*-позитивне астенозооспермичне ћелије показале умерено смањење површине нуклеуса. У нормозооспермичним узорцима, *ZIP14*-негативни сперматозоиди показали су нижи интензитет флуоресценције боје *Sytox™ Orange (Thermo Fisher Scientific)* у односу на *ZIP6*-негативне ћелије, што указује на локализоване разлике у приступачности хроматина између субпопулација дефинисаних експресијом транспортера.

Закључак Експресија *ZIP6* одражава степен сазревања и функционалну компетентност сперматозоида, док *ZIP14* показује конститутивно стабилан образац експресије. Оба транспортера могу представљати молекуларне маркере за селекцију сперматозоида високог квалитета у асистираним репродуктивним технологијама, нарочито у популацијама изложеним професионалним стресорима који нарушавају интегритет ДНК.

Кључне речи: *ZIP6*; *ZIP14*; сперматозоиди; доступност хроматина; астенозооспермија; нормозооспермија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The epidemiology and clinical characteristics of rhegmatogenous retinal detachment in a Serbian tertiary health care institution – a two-year-long retrospective study

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SUMMARY

Introduction/Objective Rhegmatogenous retinal detachment is an ophthalmic emergency which can result in vision loss. The aim of our study was to evaluate the demographic, epidemiological, and clinical characteristics and treatment methods of rhegmatogenous retinal detachment.

Methods This retrospective study was conducted at the Department of Vitreoretinal Surgery and Eye Trauma in the period between January 1, 2024, and December 31, 2025. The data included sex, age, season, onset of symptoms, presence of myopia, previous cataract surgery, as well as the method of treatment.

Results Out of 275 patients, there were 177 males (64.36%) and 98 females (35.64%). The majority of the study population was 61–80 years old (n = 140, 50.91%). The most commonly selected surgical procedure was pars plana vitrectomy (n = 226, 81.88%). Silicone oil was the preferred tamponade (n = 191, 75.2%). Best-corrected visual acuity on admission was worse than 0.1 in the majority of the affected eyes (n = 177, 64.13%), while one month after surgery nearly half (n = 130, 47.1%) achieved visual acuity between 0.1 and 0.5. Recurrent detachment occurred in 29 patients (10.32%).

Conclusion In conclusion, our study emphasizes that rhegmatogenous retinal detachment primarily affects males and individuals over 60 years of age within our region. Consequently, there is an urgent need to implement targeted public health initiatives to educate high-risk individuals on early symptoms, optimize primary ophthalmic triage, and establish efficient referral pathways to ensure timely surgical intervention and preserve functional vision.

Keywords: rhegmatogenous retinal detachment; pars plana vitrectomy; visual outcomes; epidemiology; Serbia

INTRODUCTION

Rhegmatogenous retinal detachment (RRD) is an ophthalmic emergency which can result in vision loss. It represents separation of the neurosensory retina from the retinal pigment epithelium caused by retinal breaks – tears or holes. Posterior vitreous detachment is a normal, age-related process. However, it can lead to the formation of retinal breaks in 8–16% of patients. Additionally, these breaks can progress to RRD in up to half of cases, if left untreated [1, 2].

Recent epidemiological data from 2024 estimate the mean annual incidence of RRD in Europe at 14.5 cases per 100,000 persons, with longitudinal trends pointing towards a projected doubling in incidence over the next 20 years [3].

Rhegmatogenous retinal detachment requires surgical treatment and can be managed by three main procedures: scleral buckling (SB), pars plana vitrectomy (PPV) and pneumatic retinopexy. Success rates with SB have been reported to be 66–96.7%; PPV is reported to be successful in about 71–97% of cases [4], while pneumatic retinopexy was successful in 51–82% [5]. Selection of a surgical procedure involves consideration of patients' characteristics, ocular comorbidities and characteristics of the retinal detachment itself, as well as the personal preference of the surgeon [6]. Previous studies have shown that even with appropriate and prompt treatment, many patients with RRD still need additional surgery and often do not fully recover functional vision in the affected eye [7].

The aim of our study was to evaluate the demographic, epidemiological, and clinical characteristics and treatment methods of patients hospitalized and treated for RRD at the Department of Vitreoretinal Surgery and Eye Trauma in a tertiary health care institution. These findings can assist in the early recognition, evaluation, and treatment of RRD, which contributes to the prevention of irreversible vision loss.

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METHODS

This retrospective study was conducted in the period between January 1, 2024, and December 31, 2025. The study included all patients hospitalized at the Department of Vitreoretinal Surgery and Eye Trauma for the treatment of RRD in the specified period. The patients' data were collected from the Heliant Healthcare medical system. The data included sex, age, season, onset of symptoms, presence of myopia, previous cataract surgery, as well as the method of treatment and postoperative complications.

Patients with other forms of retinal detachment (tractional and exudative) and those who presented with retinal redetachment were excluded from the study.

Patients who met the inclusion criteria underwent a complete ophthalmic examination of both eyes (best-corrected visual acuity (BCVA) measured on a Snellen chart, intraocular pressure (IOP) measurement using Goldmann applanation tonometry, anterior segment examination and indirect fundoscopy using a slit-lamp biomicroscope).

Patients were divided according to sex and into four groups according to age (≤ 40, 41–60, 61–80 and ≥ 81). Best-corrected visual acuity was analyzed at admission and one month postoperatively. According to BCVA, patients were divided into three groups (BCVA ≤ 0.1, 0.1–0.5, and ≥ 0.6).

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2016 software (Microsoft Corporation, Redmond, WA, USA), Google Sheets (Google LLC, Mountain View, CA, USA), and R version 4.3.1 (2023 The R Foundation for Statistical Computing). Descriptive data are presented as absolute and relative numbers in the form of n (%). The normality of distribution for numerical variables was assessed using the Shapiro–Wilk test. Numerical variables deviated from a normal distribution ($p < 0.05$) and were presented as medians and ranges. The Mann–Whitney U test was used to compare two independent groups, the Kruskal–Wallis test for comparisons among multiple groups, and the Wilcoxon test was used to evaluate dependent variables. Categorical variables are expressed as frequencies and percentages, and were compared using the χ^2 test. Statistical significance was established at a p-value of less than 0.05.

RESULTS

During the defined period, a total of 275 patients (276 eyes) were hospitalized for RRD. There were significantly ($p < 0.001$) more males (n = 177, 64.36%) than females (n = 98, 35.64%), with a male-to-female ratio of 1.8:1. The youngest patient was 23 years old, and the oldest was 87, with a median age of 62. There was a statistically significant difference in the distribution of patients across age groups ($p < 0.001$). The majority of the study population was 61–80 years old (n = 140, 50.91%).

The number of cases whose RRD involved the fovea (macula-off RRD) was 220 (79.71%). In the remaining 56 (20.29%) the fovea was preserved (macula-on RRD). Patients with macula-off RRD presented significantly later ($p = 0.002$) with a median delay of 5.5 days (interquartile range (IQR) 11), while in macula-on patients it was three days (IQR 5). Details regarding the age distribution of the entire study group, as well as foveal involvement among age groups, are presented in Table 1.

Table 1. Age distribution and distribution of foveal involvement in all study groups

Age (years)	n (%)	Macula-on RRD (n, %)	Macula-off RRD (n, %)
≤ 40	9 (3.27)	1 (1.79)	8 (3.63)
41–60	120 (43.64)	34 (60.71)	87 (39.55)
61–80	140 (50.91)	21 (37.5)	119 (54.09)
81 and older	6 (2.18)	0	6 (2.73)

RRD – rhegmatogenous retinal detachment

The median time from onset of symptoms until the first examination was five days (IQR 12; range: 1–120). There was a statistically significant difference in onset of symptoms between age groups ($p < 0.001$). The shortest time from symptom onset to the first examination was observed in the group of patients aged 41–60 years (four days), while the longest time was recorded in patients aged 61–80 years (seven days). The median time to presentation was seven days for women and four days for men ($p = 0.002$).

Seasonal analysis showed the highest number of RRD cases in spring (n = 82, 29.71%) and seasonal distribution was statistically uneven ($p = 0.024$).

The right eye was more frequently affected (n = 152, 55.07%). Of the 275 patients, one had bilateral eye involvement at the time of examination. The majority of the eyes with RRD were phakic (n = 198, 71.74%).

Retinal breaks were classified based on the quadrant in which they were located: superotemporal (STQ), superonasal (SNQ), inferotemporal (ITQ), and inferonasal (INQ). Most cases of RRD were caused by a superotemporal retinal break (n = 175, 55.56%). Table 2 provides comprehensive information on the affected eye (right or left), seasonality, lens status, and location of the retinal break.

In the majority of the affected eyes (n = 176, 63.77%), only one retinal break was found, while the highest number of retinal breaks was six. Table 3 summarizes the distribution of patients by the number of retinal breaks.

According to the medical history and anamnestic data collected during admission, 69 (25%) of all patients had myopia.

Intraocular pressure was measured on the day of admission and one month postoperatively. On the day of admission, the median IOP was 13 mmHg (IQR 4; range: 1–21), and one month after surgery it was 14 mmHg (IQR 5; range: 3–28), $p < 0.001$.

Best-corrected visual acuity significantly improved after treatment ($p < 0.001$). Best-corrected visual acuity on admission was worse than 0.1 in 177 (64.13%) eyes, while one month after surgery nearly half of the affected eyes

Table 2. Baseline characteristics of the entire study population, including laterality, seasonality, lens status and location of retinal break in eyes with rhegmatogenous retinal detachment

Characteristics	Number of eyes	%
Laterality		
Right	152	55.07
Left	124	44.93
Season		
Spring	82	29.71
Summer	71	25.72
Fall	48	17.39
Winter	75	27.17
Lens status		
Phakic	198	71.74
Pseudophakic	76	27.54
Aphakic	2	0.72
Quadrants		
STQ	175	55.56
SNQ	35	11.11
ITQ	78	24.76
INQ	27	8.57

STQ – superotemporal quadrant; SNQ – superonasal quadrant; ITQ – inferotemporal quadrant; INQ – inferonasal quadrant

Table 3. Number of retinal breaks found in eyes with rhegmatogenous retinal detachment

Number of retinal breaks	n	%
1	176	63.77
2	55	19.93
3	27	9.78
4	6	2.17
5	6	2.17
6	2	0.73
Dialysis	3	1.09
Macular hole	1	0.36

(n = 130, 47.1%) achieved visual acuity between 0.1 and 0.5. Details regarding visual acuity before and one month after surgery are shown in Figure 1.

At the time of the first examination, 32 (11.59%) patients had developed proliferative vitreoretinopathy (PVR). Patients with PVR presented considerably later. The median time was 30 days in patients with PVR compared with four days in patients without PVR ($p < 0.001$). The vast majority of patients with PVR (n = 30, 93.75%) presented with very poor visual acuity, which was less than 0.1. The rest of the cases with PVR (n = 2, 6.25%) had visual acuity 0.1–0.5 on admission. One month postoperatively, 25 (78.13%) patients with PVR at admission still had BCVA below 0.1, whereas seven (21.87%) of them obtained visual acuity 0.1–0.5. Out of 32 eyes with preoperative PVR, redetachment developed in six (18.75%).

In terms of treatment method, six (2.17%) of the affected eyes were managed with a non-surgical procedure – argon laser retinopexy. The most commonly selected surgical procedure was PPV, performed in 226 (81.88%) cases of RRD. Thirty-two (11.59%) underwent SB, and 12 (4.36%) were treated with pneumatic retinopexy.

Silicone oil (SO) was the preferred tamponade for RRD (n = 191, 75.2%), followed by sulfur hexafluoride (SF6) tamponade (n = 24, 9.45%), ethane hexafluoride in 1.97%

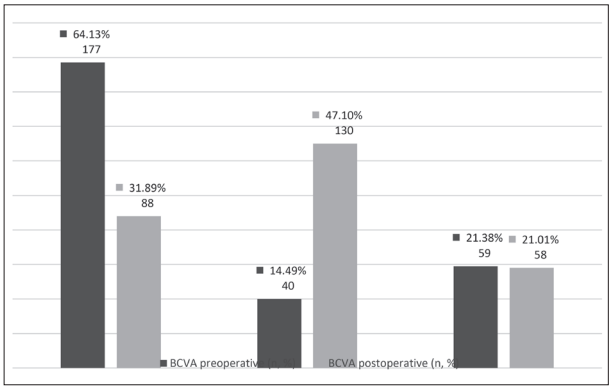


Figure 1. Best-corrected visual acuity (BCVA) of eyes with rhegmatogenous retinal detachment before surgery and one month after surgery

(n = 5) and propane octafluoride in six cases (2.36%). In 22 (68.75%) of 32 cases managed with SB, no intraocular tamponade was used. In six (18.75%) of the remaining cases, air was used and in four (12.5%), SF6 was used as an intraocular tamponade.

When SO was applied in PPV, the mean duration of oil tamponade was 5.9 ± 2.03 months. At the time of gathering data for this study and analyzing it, SO removal had been performed in 136 cases (49.29%).

In terms of postoperative complications, most study patients remained without complications (n = 187, 66.55%). The most common complications were postoperative glaucoma (n = 44, 15.66%), recurrent RRD (n = 29, 10.32%), SO in the anterior chamber (n = 10, 3.56%), and hyphema (n = 7, 2.49%). Additional complications comprised endophthalmitis, conjunctival cysts, cystoid macular edema and scleral rupture, observed in one case each. Some patients had more than one complication at the same time.

Of the 29 patients with recurrent RRD, 18 (6.55%) had redetachment under SO after PPV, four (1.45%) patients had recurrence after SO removal, and three patients (1.09%) had recurrence after SB; in one of these cases, the retina was initially attached, whereas in the other two cases the initial scleral buckling procedure had been unsuccessful.

DISCUSSION

Our study showed that males are at greater risk of RRD compared to females. This finding was consistent with the results reported by Rhyno et al. [8] in 2026, who also observed a statistically significantly higher number of male patients. The right eye was more frequently affected in most patients, in agreement with the results reported by Kasetty et al. [9]. Furthermore, several studies reported a correlation between the higher rate of RRD in men and work-related physical strain, such as lifting heavy objects. This could explain the higher incidence of RRD in males in general, considering that most of the physically demanding jobs are undertaken by males [10]. On the other hand, one Danish study did not find a higher incidence of RRD in men who perform physical work [11]. In recent studies, most patients are men, but one study shows a

predominance of female patients with a median age of 93, which can be explained by the longer life span of women. This study included an elderly population because it has been underrepresented in recent studies [12].

We analyzed seasonal variations in RRD frequency and found a decreased number of patients in fall and a higher number in spring. The results are contradictory: while one study reports a higher number of patients during warmer periods, as in our study, possibly related to increased physical activity and the influence of ultraviolet light on the vitreous and retina which initiates posterior vitreous detachment and retinal breaks [13], another reports no significant seasonal variations [14].

Myopia represents one of the major risk factors for RRD, because of the increased axial length and peripheral degenerative changes [15]. In our study, only a quarter of the entire study group had myopia, which is in contrast to results from other studies where the number of myopes with RRD was three times higher than that of non-myopes with RRD [16].

Cataract surgery is also considered a risk factor for RRD [17]; however, in our study, it was shown that twice as many patients had natural crystalline lenses, but most patients with RRD belonged to the 61–80-year age group, which is a risk factor by itself. Most studies have identified older age as a significant risk factor, which aligns with our study, demonstrating that the highest incidence of RRD is in patients 50–70 years old [18, 19]. One of the latest studies also showed similar results, with a median age of 61 years among patients with RRD [8]. Furthermore, similar findings were reported in another study performed at our clinic from 2020 to 2022 [20]. In contrast, one study from Nigeria reported that the mean age for RRD is 44 ± 17.5 years without a clearly identified reason [21].

The study has shown that PPV was the preferred surgical method for RRD in relation to SB, which concurs with the study by Rajsirisongsri et al. [22], which had the same results. These findings might be due to advances in surgical techniques – small-gauge PPV – and also to late referral to an ophthalmologist and advanced retinal detachment. In addition, since the majority of our patients were elderly and required cataract surgery, it was considered optimal to perform cataract surgery with PPV. However, in younger patients with a clear lens and uncomplicated RRD, scleral buckling would be the treatment of choice. Our study showed a high primary anatomic success rate, consistent with recent reports on RRD surgery [23]. SO was frequently used for temporary intraocular tamponade in our study. While prolonged tamponade carries risks of complications such as secondary glaucoma and cataract development in patients with a natural lens, there is also a risk of redetachment after SO removal. In contrast to our findings, one study from 2026 reported recurrent RRD in 21.1% and demonstrated that, in addition to PVR as the main factor, high myopia, pre-existing glaucoma, and systemic hypertension were also identified as contributing factors [24]. In a study done by Gisquet et al. [25], the rate of redetachment was 14.9% and SO tamponade lasted almost three months, which the authors regarded as a short time and a potential contributing factor to recurrent RRD after removal of SO. In contrast, the mean duration of SO tamponade in our study was approximately six months. Gas tamponade is another treatment option, with SF6 most frequently used for RRD without PVR, in cases with superior retinal breaks, or as an intraocular tamponade during SB.

For vitreoretinal surgeons, PVR is still difficult to overcome and is one of the most significant factors for surgical failure and poor functional outcome, with success rates decreased by more than 46.9% in cases with PVR [26]. Our study showed no significantly increased number of patients with PVR compared to the results of the previously mentioned study, but the reattachment rate in the group of patients with preoperative PVR was 18.76%, and BCVA improved by 15.62%. However, study results are contradictory: while some recent studies indicate that PVR is associated with poor visual outcomes [24, 27], another study reports no significant correlation between PVR and final BCVA [28]. Primary surgical success and the absence of a severe postoperative inflammatory reaction are considered to have the greatest impact on BCVA improvement in patients with successful retinal reattachment.

Postoperative visual acuity is a crucial indicator of surgical success, and a study by Zaletel Benda et al. [28] has demonstrated a direct relationship between preoperative and postoperative visual acuity and vice versa. The duration of symptoms seems to be closely related to postoperative BCVA, with a duration longer than seven days being associated with poor functional visual outcomes [29]. The time from the onset of symptoms until examination and treatment was longest in patients aged between 61 and 80 years in this study. This finding may be associated with age-related factors and socioeconomic conditions. Barequet et al. [27] found that macular status is important and that macula-on detachment had better anatomical and functional outcomes. In our study, a significant number of patients achieved better postoperative BCVA, even though the average duration of symptoms in the largest group of patients was longer than one week, and the majority of them had an affected macula. The majority of patients in our study had a single break, most frequently in the superior temporal quadrant, which is the reason why most of the patients had "macula-off" at the time of admission. This finding is consistent with a study reporting that more than half of patients have retinal breaks located in the superior half of the retina [30].

This study has several limitations. First, it was retrospective and based on data obtained from medical records. Second, it was performed at a single center, which may limit generalization to a larger population. In addition, only patients with primary RRD were included.

CONCLUSION

In conclusion, our study emphasizes that rhegmatogenous retinal detachment primarily affects males and individuals over 60 years of age within our region. Pars plana

vitrectomy with a six-month SO tamponade represents the predominant and anatomically effective surgical approach in our tertiary center. Most patients delay seeking ophthalmic evaluation for more than seven days, typically presenting only after macular involvement causes significant vision loss. Consequently, there is an urgent need to implement targeted public health initiatives to educate high-risk individuals on early symptoms, optimize primary ophthalmic triage, and establish efficient referral pathways to ensure timely surgical intervention and preserve functional vision.

Ethics: This study was conducted according to the tenets of the Helsinki Declaration and approved by the Hospital's Committee on Ethics (635/11).

Conflict of interest: None declared.

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Епидемиологија и клиничке карактеристике регматогене аблације мрежњаче у терцијарном здравственом центру у Србији – двогодишња ретроспективна студија

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САЖЕТАК

Увод/Циљ Регматогено одвајање мрежњаче представља ургентно стање у офталмологији које може довести до трајног губитка вида. Циљ ове студије био је да се анализирају демографске, епидемиолошке и клиничке карактеристике, као и методе лечења регматогеног одвајања мрежњаче.

Методе Спроведена је ретроспективна студија на Одељењу за ретиновитреалну хирургију и трауматологију ока у периоду од 1. јануара 2024. године до 31. децембра 2025. године. Анализирани су подаци о полу, старости, годишњем добу, почетку симптома, присуству миопије, операцији катаракте и методи лечења.

Резултати Од 275 пацијената, мушкараца је било 177 (64,36%), а жена 98 (35,64%). Већина испитаника била је старости између 61 и 80 година (*n* = 140, 50,91%). Витректомија је била најчешћа хируршка метода лечења (*n* = 226, 81,88%), а силиконско уље најчешће средство за тампонаду (*n* = 191, 75,2%). Најбоље коригована видна оштрина на пријему била је мања од 0,1 у већини захваћених очију (*n* = 177, 64,13%), док је месец дана након операције код скоро половине очију забележена видна оштрина између 0,1 и 0,5 (*n* = 130, 47,1%). Поновно одвајање мрежњаче забележено је код 29 пацијената (10,32%).

Закључак Наша студија показује да се регматогено одвајање мрежњаче на нашим просторима чешће јавља код мушкараца и особа старијих од 60 година. Сходно томе, потребно је хитно спровести циљане јавноздравствене иницијативе за едукацију високоризичних особа о раним симптомима, унапређење примарне офталмолошке тријаже и успостављање ефикасних путева упућивања како би се обезбедила правовремена хируршка интервенција и очување функционалног вида.

Кључне речи: регматогено одвајање мрежњаче; витректомија; видни исходи; епидемиологија; Србија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Postoperative complications following open and laparoscopic radical prostatectomy – a retrospective analysis using the Clavien–Dindo classification

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SUMMARY

Introduction/Objective Prostate cancer is the most commonly diagnosed malignancy in men in more than half of the world's countries and a leading cause of cancer death. Radical prostatectomy is the standard surgical treatment but carries a risk of postoperative complications. The aim of this study was to analyze postoperative complications after open and laparoscopic radical prostatectomy using the Clavien–Dindo classification.

Methods A retrospective study included 304 patients with prostate cancer treated at the University Clinical Center of Vojvodina, Clinic of Urology, from 2020 to 2024. Complications occurring within 60 days of surgery were graded using the Clavien–Dindo classification and analyzed in relation to sociodemographic, preoperative, intraoperative, and postoperative factors.

Results The mean age was 67.4 ± 5.7 years. Most complications were mild (Grade I, 47%; Grade II, 35.2%); the most frequent were blood transfusion (18.7%) and elevated serum creatinine (10.9%). Complication severity correlated significantly with body mass index ($\rho = 0.169$, $p < 0.01$), preoperative prostate-specific antigen ($\rho = 0.167$, $p < 0.01$), operative duration ($\rho = 0.719$, $p < 0.001$), and intraoperative blood loss ($\rho = 0.552$, $p < 0.001$). Sociodemographic factors showed no significant association. The difference between the open and laparoscopic approaches was not statistically significant ($p = 0.295$).

Conclusion Postoperative complications after radical prostatectomy were predominantly mild. Body mass index, prostate-specific antigen, operative duration, intraoperative blood loss, and the postoperative course were associated with greater complication severity, whereas the surgical approach was not.

Keywords: prostate cancer; prostatectomy; laparoscopy; postoperative complications; risk factors; treatment outcome

INTRODUCTION

The prostate is an encapsulated fibromuscular gland in the male pelvis whose main function is to produce seminal fluid [1]. Malignant prostate neoplasms are predominantly adenocarcinoma (90–95%), with rare mucinous, ductal, squamous, neuroendocrine, and sarcomatoid types [2].

Prostate cancer is the most commonly diagnosed cancer in men in more than half of the world's countries and the leading cause of cancer death in men in a quarter of them [3]. In 2022, an estimated 1,466,680 new cases were diagnosed globally [4]. According to the Global Cancer Observatory, in 2020 the Republic of Serbia ranked third in mortality and fourth in incidence [5]. The main non-modifiable risk factors are older age, race, and a positive family history; the prevalence of disease with a positive family history is approximately 20%, while the hereditary form ranges between 5% and 15% [6].

Current treatment modalities include active surveillance, surgery, and radiotherapy, with the choice depending on tumor characteristics, prostate-specific antigen (PSA) level, disease stage, and recurrence. Androgen deprivation therapy is used for localized, locally advanced, and metastatic disease [7]. Radical prostatectomy is the gold standard for localized prostate cancer and has been performed for more than a century [8]. There are three techniques – open, laparoscopic, and robot-assisted laparoscopic prostatectomy – and minimally invasive procedures have increasingly replaced open surgery [9]. Robot-assisted radical prostatectomy is associated with better perioperative outcomes than open and conventional laparoscopic surgery, owing to lower blood loss, lower transfusion rates, and shorter hospital stay [9]. However, a recent network meta-analysis found no significant difference between the robot-assisted, laparoscopic, and open approaches in estimated blood loss or in overall and major complication rates [10].

Despite these advances, patients remain at risk of adverse outcomes, which may depend on the surgeon's experience, technical difficulties, and individual patient characteristics [11]. Surgical site infection is among the most common complications after radical prostatectomy, and a prolonged operative time has been identified as an independent predictor of its occurrence [12]. A structured system for identifying

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the number and severity of complications is necessary to compare procedures, outcomes, and surgical quality across institutions and surgeons [13]. The Clavien–Dindo classification is increasingly used to evaluate postoperative complications but is not yet routinely applied in clinical practice.

The aim of this study was to determine the types and frequency of postoperative complications after open and laparoscopic radical prostatectomy using the Clavien–Dindo classification, and to assess the association of sociodemographic, preoperative, intraoperative, and postoperative factors with complication severity, as well as to compare the two surgical approaches.

METHODS

Study design and sample

A retrospective study included 304 patients with prostate cancer treated at the University Clinical Center of Vojvodina, Clinic of Urology, between January 1, 2020, and December 31, 2024. The following data were analyzed: sociodemographic characteristics (age, place of residence); body mass index (BMI), preoperative PSA, prostate volume, American Society of Anesthesiologists (ASA) physical status, Gleason score, and International Society of Urological Pathology (ISUP) Grade Group; type of surgery (open or laparoscopic radical prostatectomy, with or without lymphadenectomy, including conversion from laparoscopic to open); operative duration (min); intraoperative blood loss (mL); length of postoperative stay (days); time in the intensive care unit (ICU, hours); and duration of ventilatory support (hours). Postoperative events and complications were assessed within 60 days of surgery and graded using the Clavien–Dindo classification. A 60-day window was chosen because it corresponds to the institutional follow-up schedule, in which the first postoperative outpatient visit with clinical and laboratory reassessment is performed within two months of surgery; this interval captures the early complications covered by the conventional 30-day window together with events presenting somewhat later, while remaining within the period for which complete documentation was available for all patients. Data were collected from the Clinical Information System and patient medical records.

Inclusion criteria were a histopathologically confirmed diagnosis of prostate cancer, radical prostatectomy performed within the study period, and complete data permitting evaluation of complications according to the Clavien–Dindo classification. Patients without a confirmed diagnosis, those operated on outside the study period, and those with incomplete clinical or sociodemographic data were excluded.

Clavien–Dindo classification

Each complication was graded from I to V: Grade I – any deviation from the normal postoperative course not requiring pharmacological, surgical, endoscopic, or radiological treatment; Grade II – complications requiring pharmacological treatment; Grade III – complications requiring surgical, endoscopic, or radiological intervention (IIIa without and IIIb under general anesthesia); Grade IV – life-threatening complications requiring intensive care (IVa, single-organ; IVb, multiorgan dysfunction); and Grade V – death [14]. The main advantage of the classification is its simplicity; its limitations include grading according to the intervention required rather than the true severity, assessment of postoperative (not intraoperative) complications, and the inability to distinguish early from late complications [13, 15]. The classification was adapted to the most common complications after radical prostatectomy as previously described [16]; the complications assigned to each grade are presented in Table 4.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation and categorical variables as frequencies and percentages. Associations between continuous and ordinal variables were assessed using Spearman's correlation coefficient (ρ), and associations between categorical variables using the χ^2 test. Independent predictors of complication severity were identified by multivariate logistic regression (BMI, PSA, prostate volume, ASA status, Gleason score, and ISUP Grade Group). The open and laparoscopic approaches were compared using the χ^2 test and the Mann–Whitney U test. A p-value < 0.05 was considered statistically significant.

RESULTS

General characteristics of patients and surgery

Patient and surgical characteristics are summarized in Table 1. The mean age was 67.4 ± 5.7 years, and patients came from rural and urban areas in similar proportions. The mean BMI was 27.2, mean preoperative PSA 11.5 ng/mL, and mean prostate volume 47 mL. Most patients were ASA status 3 (68.8%); by ISUP Grade Group, Group 2 was most common (32.2%), followed by Group 3 (28.9%), and Group 1 was least common (3.3%).

Surgical approaches, operative duration, and blood loss are shown in Table 2 and Figures 1–3. Open radical prostatectomy with lymphadenectomy was most common, and laparoscopic radical prostatectomy with lymphadenectomy least common (5.9%). In one patient converted from laparoscopic to open surgery, an intraoperative rectal laceration occurred and was sutured. The mean operative duration was 178.6 ± 27.4 min and the mean blood loss 628.3 ± 401.5 mL. The mean hospital stay was significantly longer after open than after laparoscopic radical prostatectomy (8 ± 4 *vs.* 6.4 ± 3.4 days; t = 3.49; p < 0.001).

Almost half of the patients had Grade I complications (47%; Table 3). The most common individual

Table 1. Patient characteristics

Variable	Value
Number of patients	304
Age, years, mean (SD)	67.4 (5.7)
Place of residence, n (%)	
Rural	142 (46.7)
Urban	162 (53.3)
BMI, mean (SD)	27.2 (3.3)
PSA, ng/mL, mean (SD)	11.5 (6.3)
Prostate volume, mL, mean (SD)	47 (31.1)
ASA status, n (%)	
2	94 (30.9)
3	209 (68.8)
4	1 (0.3)
Gleason score, n (%)	
3+3	11 (3.6)
3+4	100 (32.9)
4+3	85 (28)
4+4	52 (17.1)
4+5	45 (14.8)
5+4	9 (3)
5+5	2 (0.7)
ISUP Grade Group, n (%)	
1	10 (3.3)
2	98 (32.2)
3	88 (28.9)
4	52 (17.1)
5	56 (18.4)

SD – standard deviation; BMI – body mass index; PSA – prostate-specific antigen; ASA – American Society of Anesthesiologists; ISUP – International Society of Urological Pathology

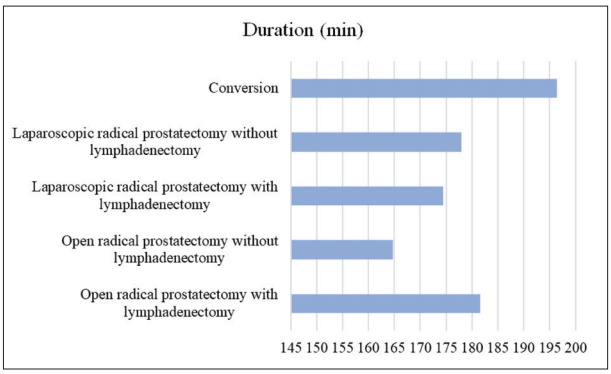


Figure 1. Mean operative duration (minutes) according to the type of radical prostatectomy; bars represent arithmetic means for five surgical subgroups: open radical prostatectomy with lymphadenectomy (n = 161), open radical prostatectomy without lymphadenectomy (n = 41), laparoscopic radical prostatectomy with lymphadenectomy (n = 18), laparoscopic radical prostatectomy without lymphadenectomy (n = 73), and procedures converted from laparoscopic to open surgery (n = 11); measures of dispersion are not displayed; the standard deviation for the whole cohort is reported in Table 2 (178.6 ± 27.4 minutes)

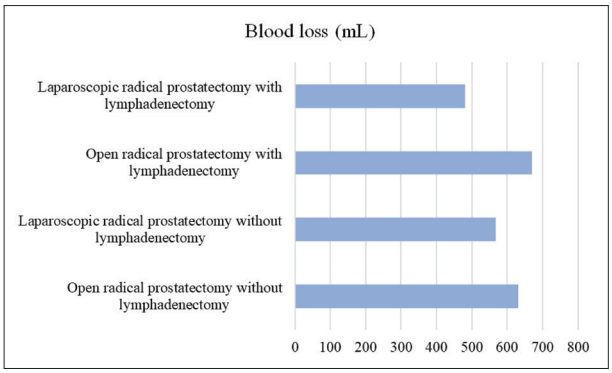


Figure 2. Mean intraoperative blood loss (mL) according to the type of radical prostatectomy; bars represent arithmetic means for four surgical subgroups: open radical prostatectomy with lymphadenectomy (n = 161), open radical prostatectomy without lymphadenectomy (n = 41), laparoscopic radical prostatectomy with lymphadenectomy (n = 18), and laparoscopic radical prostatectomy without lymphadenectomy (n = 73); measures of dispersion are not displayed; the standard deviation for the whole cohort is reported in Table 2 (628.3 ± 401.5 mL)

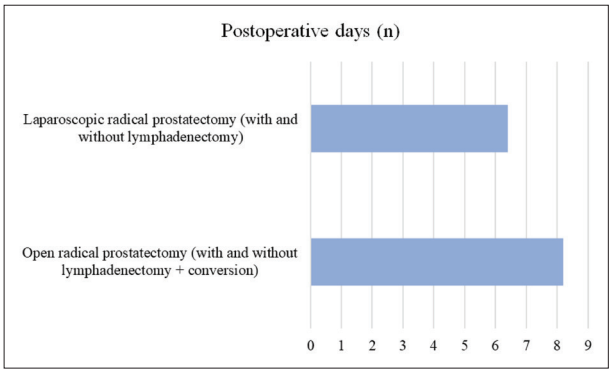


Figure 3. Mean length of postoperative hospital stay (days) according to the surgical approach; bars represent arithmetic means for open radical prostatectomy (with and without lymphadenectomy, including conversions from laparoscopic to open surgery) and for laparoscopic radical prostatectomy (with and without lymphadenectomy); measures of dispersion are not displayed; the corresponding values were 8 ± 4 days after the open and 6.4 ± 3.4 days after the laparoscopic approach (mean ± standard deviation; t = 3.49; p < 0.001)

Table 2. Surgery details

Variable	Value
Type of surgery, n (%)	
Radical prostatectomy with lymphadenectomy (open)	161 (53)
Radical prostatectomy without lymphadenectomy (laparoscopic)	73 (24)
Radical prostatectomy without lymphadenectomy (open)	41 (13.5)
Radical prostatectomy with lymphadenectomy (laparoscopic)	18 (5.9)
Conversion from laparoscopic to open (total)	11 (3.5)
Operative duration, min, mean (SD)	178.6 (27.4)
Blood loss, mL, mean (SD)	628.3 (401.5)
Postoperative stay, days, mean (SD)	7.7 (4.4)
Ventilatory support, hours, mean (SD) *	9.2 (7.2)
ICU stay, hours, mean (SD) **	19.4 (7.6)

SD – standard deviation; ICU – intensive care unit;
*refers to the five patients on ventilatory support;
**refers to the five patients treated in the ICU

Table 3. Patient distribution according to the Clavien–Dindo classification

Clavien–Dindo grade	n (%)
Grade I	143 (47)
Grade II	107 (35.2)
Grade IIIa	22 (7.3)
Grade IIIb	23 (7.6)
Grade IV	8 (2.6)
Grade V	1 (0.3)

complications were blood transfusion (Grade II, 18.7%) and elevated serum creatinine (Grade I, 10.9%); the full distribution is presented in Table 4.

Table 4. Complications according to the Clavien–Dindo classification

Complication	n (%)
Grade I	
Lymphocele / prolonged lymph secretion	14 (4.6)
Acute urinary retention (re-catheterization)	15 (4.9)
Superficial dehiscence / wound infection	10 (3.3)
Extravasation of urine	2 (0.7)
Peripheral neuropathy (obturator nerve)	2 (0.7)
Elevated serum creatinine	33 (10.9)
Glaucoma	1 (0.3)
Ileus	1 (0.3)
Lymphedema (scrotum, leg)	5 (1.6)
Transurethral catheter dislocation	1 (0.3)
Hernia (no treatment)	1 (0.3)
Allergic exanthema	1 (0.3)
Mild anemia	7 (2.3)
Nausea	20 (6.6)
Herpes zoster	1 (0.3)
Thrombophlebitis	1 (0.3)
Transient ischemic attack	1 (0.3)
Orthostatic hypotension	6 (2)
Malaise	5 (1.6)
Low-intensity pain	4 (1.3)
Sleep disturbance	6 (2)
Diarrhea	5 (1.6)
Grade II	
Blood transfusion	57 (18.7)
Urological infections (cystitis, epididymitis, etc.)	27 (8.9)
Clostridioides difficile infection	1 (0.3)
Arrhythmia requiring treatment	4 (1.3)
Phlebothrombosis	3 (1)
Pneumonia	3 (1)
Bladder tamponade	2 (0.7)
Hypertensive crisis	8 (2.6)
Exacerbation of chronic obstructive pulmonary disease	1 (0.3)
Enteritis	1 (0.3)
Wound infection (antibiotics)	1 (0.3)
Grade IIIa	
Percutaneous drainage (lymphocele, abscess, hematoma)	12 (3.9)
Endoscopic bleeding arrest (gastrointestinal)	1 (0.3)
Percutaneous nephrostomy (hydronephrosis)	3 (1)
Suprapubic catheterization (acute urinary retention)	2 (0.7)
Repeated cystoscopic catheterization	2 (0.7)
Grade IIIb	
Laparoscopic marsupialization of lymphocele	3 (1)
Laparoscopic revision (hematoma, bleeding, drain dislocation)	12 (3.9)
Early urethral stricture / bladder neck contracture	4 (1.3)
Migration of stapler into anastomosis	1 (0.3)
Hernia or fistula surgery	1 (0.3)
Surgical treatment of ileus	2 (0.7)
Complete wound dehiscence	2 (0.7)
Grade IV	
Myocardial infarction / severe arrhythmias	1 (0.3)
Stroke	1 (0.3)
Acute respiratory failure	4 (1.3)
Urosepsis	1 (0.3)
Other intensive care unit treatment (unspecified)	1 (0.3)
Grade V	
Fatal outcome	1 (0.3)

Sociodemographic factors and postoperative complications

Age was not significantly correlated with complication severity ($\rho = 0.025$, $p > 0.05$). The association between place of residence and severity was not statistically significant ($\chi^2 = 5.105$, $p = 0.403$; Table 5).

Table 5. Distribution of patients by place of residence in relation to postoperative complications

Clavien–Dindo grade	Rural, n (%)	Urban, n (%)
Grade I	60 (42.3)	83 (51.2)
Grade II	53 (37.3)	54 (33.3)
Grade IIIa	13 (9.2)	8 (4.9)
Grade IIIb	12 (8.5)	12 (7.4)
Grade IV	3 (2.1)	5 (3.1)
Grade V	1 (0.7)	0 (0)

$\chi^2 = 5.105$; $p = 0.403$

Preoperative factors

On multivariate logistic regression (Table 6), BMI and preoperative PSA contributed significantly to Grade I complications (BMI: OR 0.903, 95% CI 0.836–0.974, $p = 0.009$; PSA: OR 0.951, 95% CI 0.912–0.991, $p = 0.017$), whereas Grades II–IV could not be significantly predicted by the studied variables. Spearman correlation confirmed significant, low positive associations between complication severity and BMI ($\rho = 0.169$, $p < 0.01$) and preoperative PSA ($\rho = 0.167$, $p < 0.01$). A significant association was found with Gleason score ($\chi^2 = 64.781$, $p < 0.01$), whereas ASA status and ISUP Grade Group were not significant (Table 7).

Intraoperative factors

Operative duration ($\rho = 0.719$, $p < 0.001$) and intraoperative blood loss ($\rho = 0.552$, $p < 0.001$) showed strong, statistically significant positive correlations with complication severity.

Table 6. Multivariate logistic regression for postoperative complications by Clavien–Dindo grade

Grade	Variable	OR (95% CI)	Wald	p
I	BMI	0.903 (0.836–0.974)		0.009 *
	PSA (ng/mL)	0.951 (0.912–0.991)		0.017 *
	Prostate volume (mL)	1.001 (0.993–1.009)		0.872
	ASA status		1.188	0.552
	Gleason score		3.328	0.767
	ISUP Grade Group		0.776	0.678
II	BMI	1.069 (0.992–1.153)		0.080
	PSA (ng/mL)	1.036 (0.996–1.078)		0.081
	Prostate volume (mL)	0.998 (0.989–1.006)		0.597
	ASA status		0.230	0.891
	Gleason score		3.943	0.684
	ISUP Grade Group		0.066	0.968
IIIa	BMI	1.051 (0.918–1.204)		0.471
	PSA (ng/mL)	0.987 (0.912–1.068)		0.744
	Prostate volume (mL)	1.001 (0.985–1.018)		0.903
	ASA status		0.654	0.721
	Gleason score		4.200	0.650
	ISUP Grade Group		0.0	1.000
IIIb	BMI	1.009 (0.886–1.149)		0.894
	PSA (ng/mL)	1.026 (0.962–1.094)		0.441
	Prostate volume (mL)	1.000 (0.985–1.015)		0.978
	ASA status		0.137	0.934
	Gleason score		2.970	0.813
	ISUP Grade Group		0.0	1.000
IV	BMI	1.142 (0.918–1.421)		0.233
	PSA (ng/mL)	1.050 (0.953–1.158)		0.322
	Prostate volume (mL)	1.012 (0.992–1.033)		0.252
	ASA status		0.073	0.964
	Gleason score		5.781	0.448
	ISUP Grade Group		0.0	1.000

OR – odds ratio; CI – confidence interval; BMI – body mass index; PSA – prostate–specific antigen; ASA – American Society of Anesthesiologists; ISUP – International Society of Urological Pathology; Grade V was not analyzed (single patient);
*statistically significant

Table 7. Distribution of patients by Gleason score and Clavien–Dindo grade

Grade	3 + 3	3 + 4	4 + 3	4 + 4	4 + 5	5 + 4	5 + 5
I	8 (72.7)	50 (50)	45 (52.9)	20 (43.5)	16 (35.6)	4 (44.4)	0 (0)
II	0 (0)	35 (35)	28 (32.9)	16 (34.8)	22 (48.9)	1 (11.1)	1 (50)
IIIa	2 (18.2)	7 (7)	3 (3.5)	5 (10.9)	3 (6.7)	1 (11.1)	0 (0)
IIIb	1 (9.1)	5 (5)	7 (8.2)	4 (8.7)	3 (6.7)	2 (22.2)	0 (0)
IV	0 (0)	3 (3)	1 (1.2)	1 (2.2)	1 (2.2)	1 (11.1)	1 (50)
V	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)

Values are n (%);
$\chi^2 = 64.781$;
$p < 0.01$

Postoperative factors

Length of stay, ICU hours, and duration of ventilatory support correlated significantly and positively with complication severity (Table 8).

Comparison by surgical approach

Patients undergoing open radical prostatectomy tended to have more frequent and more severe complications than those undergoing laparoscopic surgery, except for Grade II, which was more frequent after laparoscopic surgery; however, none of the differences reached statistical significance (overall $\chi^2 = 6.120$, $p = 0.295$; Mann–Whitney U = 9525.5, Z = -0.256, $p = 0.798$; Table 9).

Table 8. Correlation between postoperative factors and complication severity (Clavien–Dindo)

Factor	Spearman's ρ	p
Postoperative days	0.323	< 0.001
Ventilatory support (hours)	0.235	< 0.001
Intensive care unit (hours)	0.235	< 0.001

Correlation significant at the 0.01 level (two-tailed)

Table 9. Comparison of complication severity between open and laparoscopic radical prostatectomy

Grade	Open, n (%)	Laparoscopic, n (%)	χ^2 (p)
I	102 (47.9)	41 (45.1)	0.205 (0.650)
II	68 (31.9)	39 (42.9)	3.34 (0.068)
IIIa	17 (8)	4 (4.4)	1.28 (0.259)
IIIb	20 (9.4)	4 (4.4)	1.19 (0.139)
IV	5 (2.3)	3 (3.3)	0.22 (0.636)
V	1 (0.5)	0 (0)	0.43 (0.513)

Overall $\chi^2 = 6.120$;
$p = 0.295$;
Mann–Whitney U = 9525.5;
Z = -0.256;
$p = 0.798$

DISCUSSION

Millions of men are diagnosed with prostate cancer each year, and prognosis varies with age, ethnicity, genetic background, and disease stage [17]. The number of men with localized disease undergoing radical prostatectomy is rising, yet mortality from metastatic disease has remained relatively unchanged [18]. Radical prostatectomy may be performed as open or minimally invasive surgery; oncological and functional outcomes depend largely on the experience of the surgeon and the center rather than on the approach alone, and the only consistent advantage of minimally invasive surgery is reduced intraoperative blood loss [19]. Beyond general surgical risks, patients should be counseled about stress urinary incontinence (5–15%) and erectile dysfunction (30–80%), the latter depending on age, nerve-sparing, and baseline function [19]. Overall outcomes also depend on baseline patient and tumor characteristics and functional status [20]. The Clavien–Dindo classification is widely used and valued for its simplicity and reproducibility, allowing comparison of surgical techniques [21].

In our study, most patients had Grade I (47%) or Grade II (35.2%) complications. Patient age was not associated with complication severity ($\rho = 0.025$), suggesting that comorbidity, physical status, and physiological reserve may be more relevant predictors [22]. BMI and preoperative PSA showed significant, low positive correlations with severity ($\rho = 0.169$ and 0.167, respectively). A significant association was found with Gleason score ($\chi^2 = 64.781$, $p < 0.01$), whereas ISUP Grade Group and ASA status were

not significant; tumor aggressiveness (ISUP Grade Group 4–5) reflects more adverse tumor biology [2], but its influence on the postoperative course requires further study. Operative duration ($\rho = 0.719$) and intraoperative blood loss ($\rho = 0.552$) correlated strongly with severity, consistent with greater physiological stress and with the literature [12, 22, 23]. Postoperative factors – length of stay, ICU time, and ventilatory support – were also significantly associated with severity, as expected. The open and laparoscopic approaches did not differ significantly, although Grade II complications were more frequent after laparoscopic surgery; this may reflect the influence of surgeon experience and individual patient factors.

Martínez-Holguín et al. [24], in a series of 518 patients, reported a shorter mean hospital stay (4 *vs*. 7.7 days) and a lower mean PSA (7 *vs*. 11.5 ng/mL) than in our cohort, with fewer transfusions (4.8%); as in our study, severe complications (≥ Grade III) were uncommon.

Dinić et al. [25] analyzed 254 patients undergoing open retropubic prostatectomy, with a similar operative duration (170 min) but greater blood loss (867 *vs*. 628.3 mL) and longer hospitalization (10.5 *vs*. 7.7 days); low-grade complications predominated, consistent with our findings.

Shabani et al. [26], in 244 patients, found no significant differences in age, BMI, or PSA between approaches – contrary to our finding of a significant association of BMI and PSA with complications – and reported significantly less blood loss and shorter hospitalization with laparoscopic surgery; in our cohort, hospital stay was likewise significantly shorter after laparoscopic surgery (6.4 *vs*. 8 days; $p < 0.001$), and Grade II complications were more frequent after the laparoscopic approach.

In a network meta-analysis of 80 studies, Kim et al. [10] found no significant difference between the robot-assisted, laparoscopic, and open approaches in estimated blood loss or in overall and major complication rates, with the longest operative time for the laparoscopic approach; this is consistent with the lack of a significant difference between the open and laparoscopic approaches in our cohort. The robot-assisted approach is not yet available at our center.

In a large cohort, Pompe et al. [16] (13,924 patients) observed predominantly mild complications after the introduction of standardized reporting (Grade I, 10.6%; Grade II, 7.9%), with severe complications (Grades IV–V) in less than 1%, consistent with our results.

Our study has limitations. Complications were recorded within 60 days of surgery, whereas most published series apply a 30-day or a 90-day window; besides limiting the assessment of long-term outcomes, this makes direct comparison with other series more difficult, as a 60-day window may capture more events than 30-day reports and fewer than 90-day reports. The absence of significant differences between approaches may partly reflect patient selection during the COVID-19 pandemic, when patients with better general health and fewer comorbidities were preferentially selected for surgery. Interobserver variability in Clavien–Dindo grading [15] and unexamined factors such as physiological reserve and surgeon experience may also have influenced the frequency and severity of complications.

CONCLUSION

Postoperative complications following open and laparoscopic radical prostatectomy were predominantly mild, with most patients classified within Clavien–Dindo Grades I and II. No statistically significant association was observed between sociodemographic factors (age and place of residence) and complication severity. In contrast, preoperative factors (BMI and PSA), intraoperative factors (operative duration and intraoperative blood loss), and postoperative factors (length of hospitalization, ICU stay, and ventilatory support) were significantly associated with greater complication severity. Although laparoscopic radical prostatectomy showed a tendency toward fewer severe complications than the open approach, this difference did not reach statistical significance.

Ethics: The study was approved by the Ethics Committee of the University Clinical Center of Vojvodina (Decision No. 00-382 of December 1, 2024). The study was conducted in accordance with the Declaration of Helsinki and conforms to applicable legal standards.

Conflict of interests: None declared.

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Постоперативне компликације након отворене и лапароскопске радикалне простатектомије – ретроспективна анализа применом Клавијен–Диндо класификације

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САЖЕТАК

Увод/Циљ Карцином простате је најчешће дијагностикован малигнитет код мушкараца у више од половине земаља света и један од водећих узрока смрти од рака. Радикална простатектомија је стандардни вид хируршког лечења, али носи ризик од постоперативних компликација. Циљ рада био је да се анализирају постоперативне компликације након отворене и лапароскопске радикалне простатектомије применом Клавијен–Диндо класификације.

Методе Ретроспективном студијом обухваћено је 304 болесника са карциномом простате лечених у Универзитетском клиничком центру Војводине, на Клиници за урологију, у периоду од 2020. до 2024. године. Компликације настале унутар 60 дана од операције градиране су према Клавијен–Диндо класификацији и анализиране у односу на социодемографске, преоперативне, интраоперативне и постоперативне факторе.

Резултати Просечна старост износила је 67,4 ± 5,7 година. Већина компликација била је блага (градус *I* – 47%; градус *II* – 35,2%); најчешће су биле трансфузија крви (18,7%) и повишене вредности серумског креатинина (10,9%). Тежина компликација значајно је корелирала са индексом телесне масе ($\rho = 0{,}169$, $p < 0{,}01$), преоперативном вредношћу антигена специфичног за простату ($\rho = 0{,}167$, $p < 0{,}01$), трајањем операције ($\rho = 0{,}719$, $p < 0{,}001$) и интраоперативним губитком крви ($\rho = 0{,}552$, $p < 0{,}001$). Социодемографски фактори нису показали значајну повезаност. Разлика између отвореног и лапароскопског приступа није била статистички значајна ($p = 0{,}295$).

Закључак Постоперативне компликације након радикалне простатектомије биле су претежно благе. Индекс телесне масе, вредност антигена специфичног за простату, трајање операције, интраоперативни губитак крви и постоперативни ток били су повезани са већом тежином компликација, док хируршки приступ није.

Кључне речи: карцином простате; простатектомија; лапароскопија; постоперативне компликације; фактори ризика; исход лечења

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Preoperative C-reactive protein predicts next-day pain after breast tumor surgery

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SUMMARY

Introduction/Objective Subclinical inflammation is increasingly recognized as a driver of postoperative pain, yet its role in breast tumor surgery is not fully defined.

The objective was to determine whether preoperative C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) predicts acute postoperative pain (APSP); to examine how surgical extent influences the perioperative inflammatory response; and to explore the relation between intraoperative fentanyl and pain intensity.

Methods In a prospective single-center cohort, 101 adults scheduled for breast surgery were stratified into four groups: mastectomy (M, n = 25), tumorectomy (T, n = 28), tumorectomy plus axillary dissection (T + A, n = 20), and benign excision (C, n = 28). Venous blood was drawn on admission (T0) and at 24 h (T24) for CRP and ESR. Pain was assessed with an 11-point Numerical Rating Scale 30 min after recovery and at 24 h; the highest 24-h score (max-APSP) was the primary pain outcome.

Results Preoperative CRP correlated with max-APSP ($p = 0.043$), whereas ESR did not ($p = 0.11$). Baseline CRP and ESR, and their 24-hour increments, rose stepwise with surgical magnitude, peaking after mastectomy (baseline CRP 7.1 ± 1.9 mg/L; T24 27.5 ± 3.6 mg/L; both $p < 0.01$ *vs.* other groups). Intraoperative fentanyl correlated with CRP at 24 hours ($r = 0.33$, $p = 0.001$) and with max-APSP ($r = 0.28$, $p = 0.005$). Despite these biological gradients, mean max-APSP remained mild to moderate across groups.

Conclusion Presurgical systemic inflammation, reflected by CRP, independently predicts early pain after breast tumor surgery. Surgical magnitude amplifies inflammatory markers, and greater fentanyl exposure parallels both inflammation and pain. Routine perioperative CRP may help identify high-risk patients for targeted anti-inflammatory and opioid-sparing analgesic strategies to reduce acute pain and potentially mitigate chronic pain development.

Keywords: acute postoperative pain; breast tumor surgery; C-reactive protein; erythrocyte sedimentation rate

INTRODUCTION

Breast cancer surgery is among the most frequently performed oncological procedures worldwide. Each year, more than two million women are diagnosed with breast cancer, with surgery remaining a core treatment modality [1, 2]. Despite advances in anesthetic and analgesic techniques, acute postoperative pain (APSP) remains common; observational data suggest that up to 60% of patients report severe pain within the first 24 hours, and inadequate early control is associated with a higher risk of persistent postsurgical pain (PPSP) [3, 4]. Unrelieved APSP impairs shoulder mobility, delays hospital discharge, and independently predicts poorer health-related quality of life, reinforcing the need for early risk stratification and procedure-specific, opioid-sparing multimodal pathways [4].

A growing body of translational research links perioperative systemic inflammation to nociceptive sensitization. Recent neurobiological evidence indicates that perioperative tissue injury can activate immune–neuronal and glial pathways, promoting proinflammatory mediator release, peripheral sensitization and central sensitization [5]. In parallel, experimental and clinical studies have associated circulating inflammatory markers, particularly C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR), with heightened pain sensitivity, although the strength and timescale of these associations vary across settings [6, 7]. CRP, an acute-phase reactant predominantly regulated by interleukin-6, is a pragmatic index of short-term inflammatory flux, whereas ESR, though widely available, is less specific and may reflect chronic processes [8]. In mastectomy cohorts, higher CRP on postoperative day 1, alongside acute pain severity, has been associated with later PPSP/chronic postsurgical pain, supporting the premise that early inflammatory load shapes pain trajectories [9, 10].

Breast surgery provides a clinically relevant model to explore these relationships. Thus, the procedures share local tissue characteristics yet differ markedly in surgical scope – from

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breast-conserving tumorectomy to mastectomy with axillary clearance, creating a natural gradient of tissue trauma and systemic inflammatory response [11]. Contemporary evidence supports regional analgesia as an important component of opioid-sparing postoperative pain management after breast cancer surgery [12]. Existing literature has extensively examined CRP in breast cancer, including its prognostic value across patient subgroups [13]. Moreover, prior reports seldom adjust for intraoperative opioid dose, although experimental and translational evidence suggests that opioid exposure may paradoxically amplify postoperative nociception through neuroinflammatory mechanisms consistent with opioid-induced hyperalgesia [14].

To address these gaps, we conducted a prospective, single-center observational study of patients undergoing breast tumor surgery. We tested three prespecified hypotheses: (i) higher preoperative CRP and ESR are associated with greater APSP intensity at 24 h; (ii) postoperative inflammatory marker levels rise in proportion to surgical extent; and (iii) intraoperative fentanyl dose correlates positively with APSP intensity at 24 h. By clarifying how routine inflammatory biomarkers relate to early pain outcomes across different surgical magnitudes, our findings aim to inform risk-stratified analgesic planning and to stimulate future interventional trials that target perioperative inflammation in breast cancer patients.

METHODS

Study design and participants

A prospective, single-center observational study was conducted at the Department of Oncological Surgery, Bežanijska Kosa University Hospital Medical Center, Belgrade, Serbia, between August 1 and November 30, 2022. The study was approved by the Institutional Ethics Committee of the Bežanijska Kosa Clinical Hospital Center (protocol No. 4831/1, 22.07.2022). All participants provided written informed consent. The investigation complied with the Declaration of Helsinki and followed General Data Protection Regulation-compliant data-protection procedures.

Consecutive adult patients (≥ 18 years) scheduled for elective breast tumor surgery were screened. Inclusion criteria were: histologically confirmed breast tumor (benign or malignant), physical status I–III, planned postoperative ward admission, and written informed consent. Exclusion criteria comprised incomplete medical records, pre-existing breast pain on the day of surgery, withdrawal of consent at any time, locally advanced or metastatic disease (stage IV), allergy to anesthetic agents, or intra-/early postoperative complications requiring protocol deviation. After screening 142 patients, 101 met all criteria and were enrolled. According to surgical extent and final histology, they were stratified into four predefined groups: 1) Mastectomy (M) – malignant, entire breast removed (n = 25); 2) Tumorectomy (T) – malignant, tumor excision only (n = 28); 3) Tumorectomy + axillary dissection (T + A) – malignant, tumor excision with axillary clearance (n = 20); 4) Benign excision (C) – benign lesion removed (n = 28).

Methods

General anesthesia was standardized in all study groups. Anesthesia was induced with propofol (1.5 mg/kg intravenously) and rocuronium bromide (0.9–1.2 mg/kg intravenously) to facilitate endotracheal intubation. Anesthesia was maintained with sevoflurane in an oxygen/air mixture, with the end-tidal concentration adjusted according to routine clinical practice to maintain an adequate depth of anaesthesia. Intraoperative analgesia consisted of fentanyl administered intravenously and titrated according to the patient's clinical response and the extent of the surgical procedure. Additional fentanyl was titrated at the anesthetist's discretion and the total intraoperative dose was recorded (50 μg/mL solution).

Venous blood was drawn on admission (T_0) and at 24 h post-surgery (T_{24}). ESR (mm/h) and CRP (mg/L) were analyzed in the hospital laboratory using standardized turbidimetric assays.

Acute postoperative pain in the surgical breast was measured with an 11-point Numerical Rating Scale (NRS; 0 = no pain, 10 = worst imaginable) – a tool validated for postoperative settings. Scores were obtained at T_0, 30 min after arrival in the recovery room ($T_{0.5}$), and at T_{24}. For analysis, the highest score within the first 24 h was designated the maximal acute postoperative pain (max-APSP). Between the scheduled pain assessments (30 minutes and 24 hours after surgery), postoperative analgesia consisted of non-steroidal anti-inflammatory drugs, administered according to the institutional postoperative pain protocol and routine clinical assessment by the nursing staff. The same postoperative analgesic strategy was applied to all study groups.

Primary outcome: association between preoperative CRP/ESR and max-APSP. Secondary outcomes: (i) relationship between surgical extent and postoperative CRP/ESR; (ii) correlation between intraoperative fentanyl dose and maximal acute postoperative pain.

Statistical analysis

Continuous variables are reported as means ± standard errors of the mean (SEM) with ranges, and categorical variables as counts (percentages). Normality was assessed with the Shapiro–Wilk test and inspection of quantile–quantile plots; homogeneity of variances with Levene's test. Between-group comparisons used one-way analysis of variance (ANOVA) when assumptions were met; post hoc tests were selected a priori according to variance diagnostics: least significant difference (LSD) or Bonferroni when variances were homogeneous, and Games–Howell when they were not. Associations between continuous measures were examined using Pearson's correlation coefficient (r) after checking linearity and distributional assumptions. To assess whether preoperative CRP was independently

associated with maximum 24-hour APSP, a multivariable linear regression analysis within the general linear model framework was performed. Surgical group was entered as a fixed categorical factor, whereas preoperative CRP, preoperative ESR, intraoperative fentanyl dose, age and body mass index (BMI) were entered as covariates. All tests were two-sided with $\alpha = 0.05$. Analyses were performed using IBM SPSS Statistics, Version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Participants' baseline characteristics

The patients who met all eligibility criteria were enrolled (N = 101). Almost the entire cohort was female (100/101, 99%). Mean age was 56.4 ± 1.7 years (range 19–88) and differed across surgical-extent strata: benign excision (C) patients were significantly younger than all malignancy groups (ANOVA F = 25.622, p < 0.001). The four predefined groups comprised 25 mastectomies (M) – mean age 61.96 ± 2.56 years (32–81); 28 tumorectomies (T) 65.93 ± 2.48 years (41–88); 20 tumorectomies with axillary dissection (T + A) 61.76 ± 2.43 years (38–76); and 28 benign excisions (C) 38.07 ± 2.79 years (19–71) (Figure 1A).

The mean tumor size across all patients was 23.45 ± 1.35 mm (range 8–98). By group, mean sizes were as follows: C, 21.42 ± 1.69 mm (10–47); M, 28.58 ± 3.82 mm (10–98); T, 21.11 ± 1.58 mm (8–40); and T + A, 23.2 ± 3.56 mm (10–80). There was no statistically significant difference in tumor size between the groups (ANOVA: F = 1.684; p > 0.05) (Figure 1B).

The mean BMI for the cohort was 25.84 ± 0.46 kg/m² (range 15.92–38.54). By group, mean BMI values were as follows: C, 23.31 ± 0.88 kg/m² (15.92–34.89); M, 26.52 ± 0.87 kg/m² (20.2–36.25); T, 27.56 ± 0.91 kg/m² (18.29–38.54); and T + A, 26.11 ± 0.82 kg/m² (17.72–32.83). Patients with malignant breast tumors who underwent tumor excision (T) had a significantly higher BMI than those who underwent excision of a benign lesion (C) (ANOVA: F = 4.634; p < 0.05) (Figure 1C).

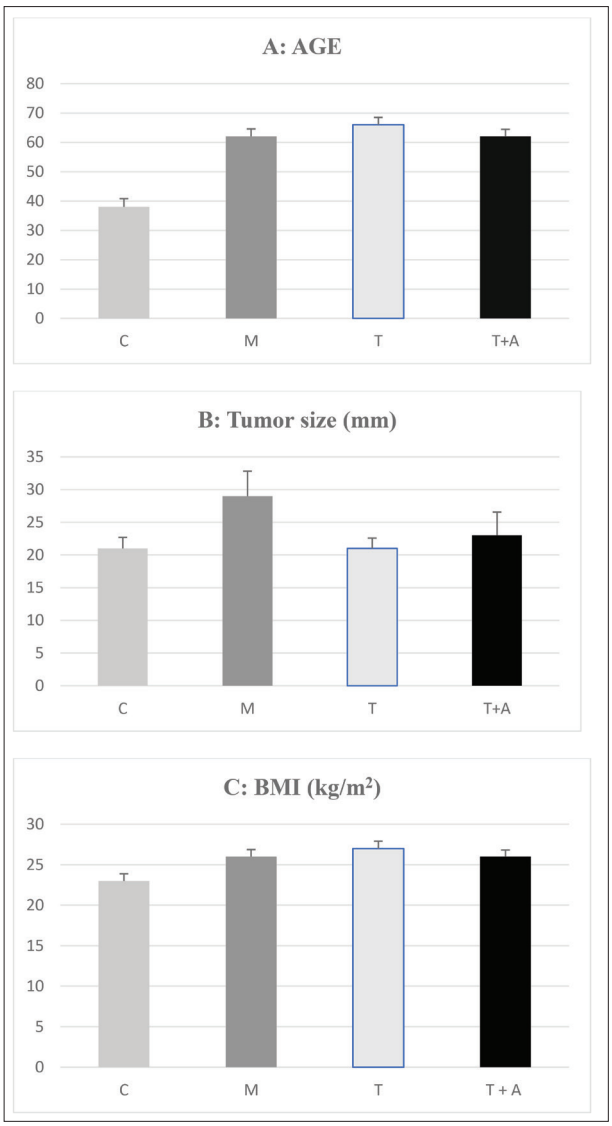


Figure 1. Baseline characteristics by surgical group; (A) age (years); one-way ANOVA with Bonferroni post hoc testing, p < 0.010; (B) tumor size (mm); one-way ANOVA, F = 1.684, p > 0.050; (C) body mass index (kg/m²); one-way ANOVA with Bonferroni post hoc testing, p < 0.050; data are mean ± SEM;
C – benign lesion excision; T – malignant tumorectomy; T + A – malignant tumorectomy with axillary dissection; M – mastectomy; significance: p < 0.050

Primary outcome – association between preoperative inflammation and 24-hour pain

In analyses of patients undergoing surgical excision of a breast tumor, a statistically significant correlation was observed between admission CRP and the maximum intensity of acute postoperative pain at 24 hours (Pearson's r = 0.246; 95% confidence interval (CI) 0.053–0.421; p < 0.05; Table 1). Admission CRP also correlated significantly with CRP at 24 hours (r = 0.574; 95% CI 0.426–0.692; p < 0.001), with the ESR at admission (r = 0.553; 95% CI 0.401–0.675; p < 0.001) and at 24 hours (r = 0.539; 95% CI 0.384–0.664; p < 0.001), as well as with the intraoperative fentanyl dose (r = 0.232; 95% CI 0.038–0.409; p < 0.05).

In addition to its positive correlation with admission CRP, the intraoperative fentanyl dose correlated significantly with CRP at 24 hours (r = 0.325; 95% CI 0.138–0.489; p = 0.001), with ESR at admission (r = 0.256; 95% CI 0.064–0.430; p = 0.01), and with the maximum intensity of acute postoperative pain at 24 hours (r = 0.279; 95% CI 0.088–0.450; p < 0.01) (Table 1).

To address potential confounding, a multivariable linear regression analysis within the general linear model framework was performed with maximum 24-hour APSP as the dependent variable. Surgical group was entered as a fixed categorical factor, while preoperative CRP, preoperative ESR, intraoperative fentanyl dose, age and BMI were entered as covariates. The overall model was statistically significant (F = 2.860, p = 0.007; $R^2 = 0.199$; adjusted $R^2 = 0.130$). Preoperative CRP remained independently associated with maximum 24-hour APSP after adjustment for surgical group, preoperative ESR, intraoperative

Table 1. Correlations of CRP at admission and intraoperative fentanyl dose with inflammatory markers and pain outcomes

Variable	Admission CRP r (95% CI)	Admission CRP (p)	Intraoperative fentanyl r (95% CI)	Intraoperative fentanyl (p)
Admission CRP	–	–	0.232* (0.038–0.409)	0.019
CRP at 24 h (mg/L)	0.574** (0.426–0.692)	< 0.001	0.325** (0.138–0.489)	0.001
ESR at admission (mm/h)	0.553** (0.401–0.675)	< 0.001	0.256** (0.064–0.430)	0.010
ESR at 24 h (mm/h)	0.539** (0.384–0.664)	< 0.001	0.160 (0.037–0.345)	0.109
APSP at 30 min (NRS 0–10)	0.062 (0.013–0.154)	0.536	0.150 (0.047–0.336)	0.133
APSP at 24 h (NRS 0–10)	0.246* (0.053–0.421)	0.043	0.279** (0.088–0.450)	0.005
Intraoperative fentanyl dose (mL; 50 µg/mL)	0.232* (0.038–0.409)	0.019	–	–

Pearson's correlation (two-tailed);
*p < 0.050;
**p < 0.010;
CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; APSP – acute postoperative pain; NRS – numerical rating scale

fentanyl dose, age and BMI (p = 0.014). Intraoperative fentanyl dose was also independently associated with maximum 24-hour APSP (p = 0.007), whereas preoperative ESR, age, BMI and surgical group were not independently associated with this outcome.

Inflammatory marker levels in relation to surgical extent in breast tumor surgery

The mean CRP on admission for the whole cohort was 3.75 ± 0.55 mg/L (range 0.6–36.2). By group, mean admission CRP values were as follows: C, 1.96 ± 0.37 mg/L (0.6–8.5); M, 7.08 ± 1.86 mg/L (0.6–36.2); T, 3.46 ± 0.73 mg/L (0.6–14.5); and T + A, 2.5 ± 0.39 mg/L (0.6–7.3). Between-group comparisons showed that patients undergoing mastectomy (M) had significantly higher circulating CRP on admission than those who underwent tumor excision (T) (ANOVA, post hoc LSD, p < 0.05), tumor excision with axillary dissection (T + A) (ANOVA, post hoc LSD, p < 0.01), and excision of a benign lesion (C) (ANOVA, post hoc LSD, p < 0.01) (Figure 2A).

The mean CRP at 24 hours postoperatively for the whole cohort was 14.17 ± 1.41 mg/L (range 0.6–82.4). By group, mean postoperative CRP values were as follows: C, 5.22 ± 1.14 mg/L (0.6–23.4); M, 27.54 ± 3.61 mg/L (3.9–82.4); T, 11.96 ± 1.8 mg/L (0.9–34.9); and T + A, 13.11 ± 2.19 mg/L (0.9–43.6). Comparisons across groups, stratified by surgical extent, showed that patients who underwent mastectomy (M) had significantly higher circulating CRP at 24 hours than those who underwent tumor excision (T) (ANOVA, post hoc Games–Howell, p < 0.05), tumor excision with axillary dissection (T + A) (ANOVA, post hoc Games–Howell, p < 0.05), and excision of a benign lesion (C) (ANOVA, post hoc Games–Howell, p < 0.001) (Figure 2B).

The mean ESR on admission was 23.62 ± 1.75 mm/h (range 2–75). By group, mean admission ESR values were as follows: C, 14.32 ± 2.32 mm/h (2–49); M, 33.48 ± 3.86 mm/h (9–75); T, 24.71 ± 3.06 mm/h (2–60); and T + A, 22.8 ± 3.98 mm/h (2–73). Between-group comparisons showed significantly higher ESR on admission in patients who underwent mastectomy (M) compared with those who had excision of a benign lesion (C) (ANOVA: F = 6.050; post hoc Bonferroni, p < 0.001) (Figure 3A).

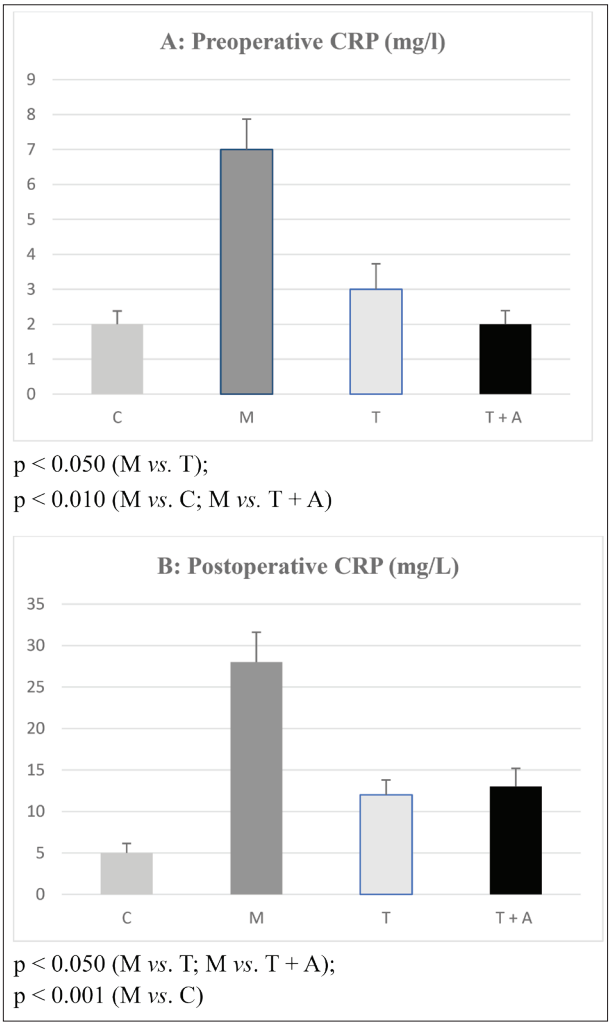


Figure 2. C-reactive protein (CRP) by surgical group; (A) admission CRP (mg/L); one-way ANOVA with LSD post hoc: p < 0.05 (M *vs.* T); p < 0.01 (M *vs.* C; M *vs.* T + A); (B) CRP at 24 h (mg/L); one-way ANOVA with Games–Howell post hoc: p < 0.05 (M *vs.* T; M *vs.* T + A); p < 0.001 (M *vs.* C); data are mean ± SEM;
C – benign lesion excision; T – malignant tumorectomy; T + A – malignant tumorectomy with axillary dissection; M – mastectomy; significance: p < 0.050 (*), p < 0.010 (**), p < 0.001 (***)

At 24 hours after surgery, the cohort's mean ESR was 20.09 ± 1.55 mm/h (range 2–64). Across groups, postoperative means were as follows: C, 11.46 ± 1.61 mm/h (2–31); M, 26.28 ± 3.48 mm/h (2–64); T, 24.71 ± 3.06 mm/h (2–60); and T + A, 18 ± 3.22 mm/h (2–55). Comparative

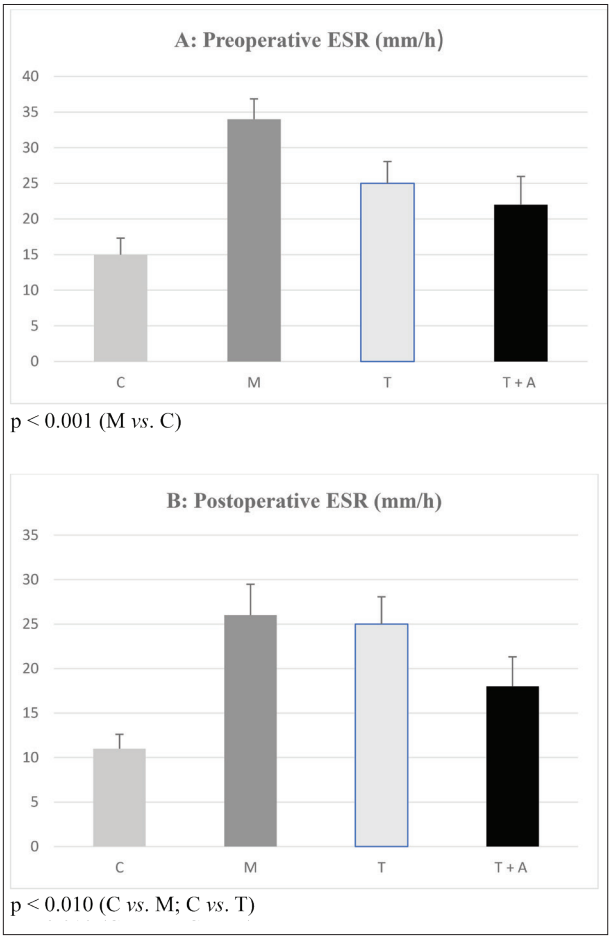


Figure 3. Erythrocyte sedimentation rate (ESR) by surgical group; (A) admission ESR (mm/h); one-way ANOVA with Bonferroni post hoc: $p < 0.001$ (M *vs*. C); (B) ESR at 24 h (mm/h); one-way ANOVA with Games–Howell post hoc: $p < 0.010$ (C *vs*. M; C *vs*. T); data are mean ± SEM;
C – benign lesion excision; T – malignant tumorectomy; T + A – malignant tumorectomy with axillary dissection; M – mastectomy; significance: $p < 0.010$ (**), $p < 0.001$ (***)

analysis by surgical extent indicated significantly lower postoperative ESR in the benign excision group (C) than in the mastectomy group (M) (ANOVA, post hoc Games–Howell, $p < 0.01$) and the tumor excision group (T) (ANOVA: $F = 5.863$; post hoc Games–Howell, $p < 0.01$) (Figure 3B).

Intraoperative fentanyl dose and acute postoperative pain

There was a statistically significant positive correlation between intraoperative fentanyl dose and the maximum intensity of acute postoperative pain at 24 h (Pearson's $r = 0.279$; 95% CI 0.088–0.450; $p < 0.01$; Table 1). For the cohort overall, the mean intraoperative fentanyl volume was 5.47 ± 0.19 mL (range 0.5–10). By group, the means were as follows: C, 4.17 ± 0.24 mL (2–8); M, 7.28 ± 0.3 mL (5–10); T, 4.52 ± 0.32 mL (0.5–7); and T + A, 6.35 ± 0.27 mL (4–8). Patients in the benign excision group (C) received significantly less fentanyl than those who underwent mastectomy (M) (ANOVA, post hoc Games–Howell, $p < 0.001$) and those who had tumor excision with

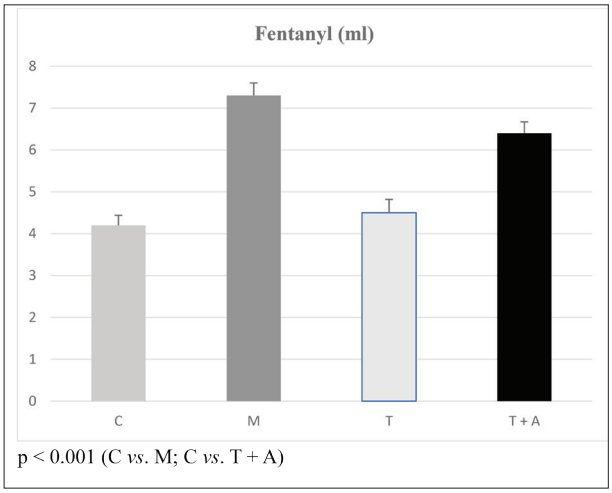


Figure 4. Intraoperative fentanyl by surgical group; mean intraoperative fentanyl volume (mL; 50 μg·mL^{-1} solution); one-way ANOVA with Games–Howell post hoc: $p < 0.001$ (C *vs*. M; C *vs*. T + A); data are mean ± SEM;
C – benign lesion excision; T – malignant tumorectomy; T + A – malignant tumorectomy with axillary dissection; M – mastectomy; significance: $p < 0.001$ (***)

axillary dissection (T + A) (ANOVA: $F = 26.544$; post hoc Games–Howell, $p < 0.001$) (Figure 4).

The mean maximum postoperative pain intensity within 30 minutes ($T_{0.5}$) of surgery for the cohort was 2.56 ± 0.21 (range 0–8). By group, the means were as follows: C, 2.11 ± 0.37 (0–8); M, 1.92 ± 0.32 (0–5); T, 3.35 ± 0.48; and T + A, 2.9 ± 0.46 (0–8). Between-group comparison showed a significant difference at 30 minutes, with patients who underwent excision of a malignant tumor (T) reporting higher maximum pain intensity than those with excision of a benign lesion (C) (ANOVA: $F = 2.786$; post hoc LSD, $p < 0.05$) (Figure 5A).

The mean maximum postoperative pain intensity within 24 hours (T_{24}) for the cohort was 2.17 ± 0.21 (range 0–8). By group, the means were: C, 1.5 ± 0.31 (0–5); M, 2.16 ± 0.39 (0–6); T, 2.35 ± 0.37 (0–5); and T + A, 2.85 ± 0.59 (0–8). There were no statistically significant differences between groups in maximum pain intensity within 24 hours of surgery (ANOVA: $F = 1.810$; $p = 0.150$) (Figure 5B).

DISCUSSION

We set out to examine whether routine inflammatory markers and intraoperative opioid exposure relate to early pain after breast tumor surgery across different procedures. In this single-centre prospective cohort, we observed three robust signals. First, higher preoperative CRP predicted more intense pain 24 h after breast tumor surgery, whereas ESR did not. Second, CRP and ESR rose in proportion to surgical magnitude. Thus, patients undergoing mastectomy exhibited the highest baseline and postoperative CRP and ESR values, followed by those receiving axillary dissection, tumorectomy, and benign excision. Third, the total intraoperative fentanyl dose correlated positively with both

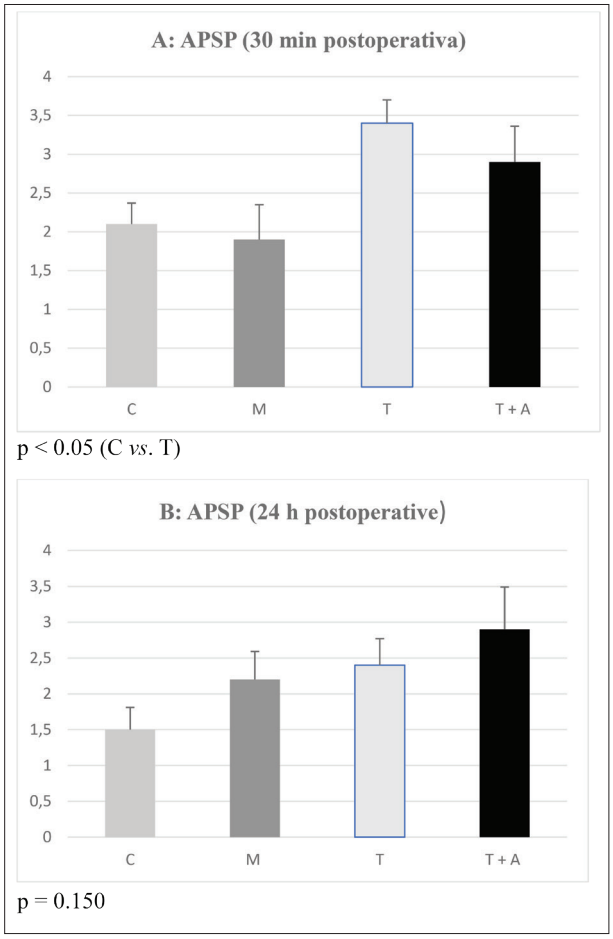


Figure 5. Maximum postoperative pain by surgical group; (A) maximum pain intensity at 30 minutes [numerical rating scale (NRS), 0–10]; one-way ANOVA with LSD post hoc: $p < 0.050$ (C *vs.* T); (B) maximum pain intensity within 24 h (NRS, 0–10); no between-group differences (one-way ANOVA, $p = 0.150$); data are mean ± SEM;
C – benign lesion excision; T – malignant tumorectomy; T + A – malignant tumorectomy with axillary dissection; M – mastectomy;
significance: $p < 0.050$

inflammatory load and 24-hour pain intensity, suggesting a neuroimmune opioid–pain feedback loop. Although mean numerical rating scale scores at 24 hours were generally mild, up to 40% of individuals still reported clinically relevant pain (NRS ≥ 4), confirming that breast surgery, even in the era of enhanced recovery, remains a procedure with substantial nociceptive burden.

Our findings align with growing evidence that low-grade systemic inflammation primes postoperative pain. Associations between perioperative CRP dynamics and acute postoperative pain have been demonstrated in other surgical populations, including total knee arthroplasty [15]. Evidence specific to breast surgery remains limited, with signals reported in mastectomy cohorts and studies linking systemic inflammation to early postoperative recovery [9, 10]. CRP generally outperforms ESR as a short-term index of inflammatory flux, consistent with its biology, while ESR is slower and more influenced by chronic processes (e.g., anemia, age) [8]. Our mastectomy subgroup showed a four-fold higher mean CRP at 24 hours than the benign excision subgroup, reinforcing the concept that tissue load predicts systemic inflammation. The gradient we observed in inflammatory response across surgical extent mirrors prior evidence that postoperative CRP kinetics vary according to the type and magnitude of surgical trauma [16]. Despite such biological gradients, early pain scores did not differ between groups at 24 hours. Two non-mutually exclusive explanations are plausible. First, effective multimodal analgesia may have equalised pain perception; second, the NRS, while valid and practical, captures only intensity, not qualitative dimensions such as movement-evoked pain [17]. Future studies incorporating quantitative sensory testing and breast surgery-specific patient-reported outcome measures may reveal more nuanced group differences [18].

Mechanistic insights

The breast cancer microenvironment is characterized by tumor-associated macrophages, fibroblasts and cytokines that elevate circulating CRP and other acute-phase reactants [19]. High preoperative CRP in mastectomy patients therefore likely reflects both tumor-driven inflammation and subclinical systemic inflammation secondary to comorbidities (obesity, metabolic syndrome) [20]. Tissue trauma may amplify this inflammatory state through the release of damage-associated molecular patterns and activation of inflammatory signalling pathways, including nuclear factor κB, within the tumor and perioperative immune microenvironment [21].

Activated nociceptors express pattern-recognition receptors; IL-1β and tumor necrosis factor trigger ion-channel phosphorylation that lowers firing thresholds, while Fcγ-receptor signaling engaged by CRP–immune complexes at the neuroimmune interface can enhance excitability. Hence, patients entering theatre with high CRP may begin their postoperative course with a "primed" nociceptive system, explaining the correlation we observed.

Preclinical and translational data suggest that opioid exposure can interact with microglial and neuroinflammatory pathways, increasing proinflammatory signalling and potentially contributing to paradoxical postoperative pain amplification [22]. These mechanisms are concordant with experimental and translational evidence implicating neuroinflammatory and inflammasome pathways in opioid-induced hyperalgesia and tolerance [14]. In our cohort, higher fentanyl requirements paralleled CRP and ESR elevations and predicted greater 24-h pain, supporting this paradigm and suggesting that opioid sparing may attenuate neuroimmune sensitization.

Clinical implications

Routine perioperative CRP measurement could be an inexpensive tool for risk stratification. Patients whose CRP exceeds a yet-to-be-defined threshold might benefit from intensified, opioid-sparing multimodal analgesia, including regional techniques and non-opioid analgesic adjuncts where appropriate [23].

Persistent inflammation is strongly linked to chronic pain; after breast cancer treatment, clinically relevant

shoulder or chest-wall pain remains common months after surgery [24]. Identifying a high-inflammation phenotype preoperatively may offer an opportunity to intervene before the transition to chronic pain occurs. Longer-term follow-up of this cohort will determine whether baseline CRP adds prognostic value to existing chronic pain prediction models.

Our data also prompt re-evaluation of routine opioid dosing in breast surgery. Adoption of regional anesthesia (paravertebral or pectoral nerve blocks) and adjuvants that reduce systemic opioid exposure may diminish both inflammation and hyperalgesic potential, ultimately improving short- and long-term outcomes [12, 14, 23].

Limitations

Strengths of this study include a prospective design, simultaneous measurement of two biomarkers and inclusion of a benign-tumor control group, enabling separation of tumor-driven from surgery-driven inflammation. Limitations comprise single-center scope, modest sample size per stratum and reliance on a unidimensional pain scale. The exclusion of regional blocks, while methodologically necessary to isolate the inflammation–pain axis, limits generalizability to settings where such analgesia is standard. Finally, we did not measure pro- and anti-inflammatory cytokines or genetic polymorphisms that might refine mechanistic understanding.

CONCLUSION

Proinflammatory status, captured by CRP but not ESR, is an independent determinant of early pain after breast tumor surgery. Surgical magnitude and intraoperative opioid dose further modulate the inflammatory–pain axis. Routine CRP assessment may guide personalized analgesic strategies and offers a biomarker target for trials aimed at preventing persistent postsurgical pain.

Future studies should establish and externally validate simple, bedside CRP thresholds that predict clinically meaningful 24–48 h pain, and test whether dampening perioperative inflammation, via optimized multimodal care and selective anti-inflammatory strategies (e.g., perioperative corticosteroids or IL-6 pathway modulation), reduces acute pain and subsequent persistence. Prediction models ought to incorporate psychophysical and psychosocial measures alongside biology to reflect the broader pain network. Finally, randomized trials comparing opioid-sparing *vs.* opioid-rich anesthesia, with immune profiling and rigorous pain phenotyping, are needed to clarify the opioid–cytokine interface and its impact on short- and longer-term outcomes.

Ethics: This study was approved by the Institutional Ethics Committee of the Bežanijska Kosa Clinical Hospital Centre (protocol No. 4831/1, 22.07.2022). All participants provided written informed consent prior to enrolment. Consent for publication of anonymized study data was obtained.

Conflict of interest: None declared.

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Преоперативни *C*-реактивни протеин предвиђа бол првог постоперативног дана након операције тумора дојке

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САЖЕТАК

Увод/Циљ Субклиничка инфламација је важан фактор у настанку постоперативног бола, али њена улога у хирургији тумора дојке није потпуно разјашњена.

Циљ рада био је да се испитају вредности преоперативног *C*-реактивног протеина (*CRP*) или седиментације еритроцита (*SE*) у предикцији акутног постоперативног бола (*APSP*), утицај обима хируршке интервенције на периоперативни инфламаторни одговор, као и повезаност интраоперативне примене фентанила са интензитетом бола.

Методе Проспективна, једноцентрична кохортна студија обухватила је 101 одраслог болесника код којег је планирана операција дојке. Болесници су били подељени у четири групе: мастектомија (*M*, *n* = 25), туморектомија (*T*, *n* = 28), туморектомија са аксиларном дисекцијом (*T* + *A*, *n* = 20) и ексцизија бенигних промена (*C*, *n* = 28). Венска крв је узимана при пријему (*T*0) и након 24 сата (*T*24). Бол је процењиван помоћу нумеричке скале (0–10) 30 минута након буђења из анестезије и након 24 сата; највиша вредност бола у прва 24 сата (*max-APSP*) била је примарни исход.

Резултати Преоперативни *CRP* био је у корелацији са *max-APSP* ($p = 0,043$), док *SE* није показала значајну повезаност ($p = 0,11$). Почетне вредности *CRP*-а и *SE*, као и њихов пораст након 24 сата, расли су пропорционално обиму хируршке интервенције, а највише вредности забележене су код мастектомије (почетни *CRP* 7,1 ± 1,9 *mg/L*; *T24* 27,5 ± 3,6 *mg/L*; оба $p < 0,01$ у односу на остале групе). Интраоперативна примена фентанила била је у корелацији са *CRP*-ом након 24 сата ($r = 0,33$, $p = 0,001$) и са *max-APSP* ($r = 0,28$, $p = 0,005$).

Закључак Преоперативна системска инфламација представља независан предиктор раног постоперативног бола након хирургије тумора дојке. Обим интервенције појачава инфламаторни одговор, док већа примена фентанила прати већи степен инфламације и интензитет бола. Рутинско одређивање *CRP*-а у периоперативном периоду може помоћи у идентификацији болесника са високим ризиком и омогућити примену циљаних антиинфламаторних аналгетских стратегија и стратегија којима се смањује употреба опиоида, ради смањења акутног бола и потенцијалне превенције хроничног бола.

Кључне речи: акутни постоперативни бол; хирургија тумора дојке; *C*-реактивни протеин; седиментација еритроцита

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Barriers to accessing healthcare systems faced by individuals with autism – a study across four European countries

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SUMMARY

Introduction/Objective Individuals with autism face significant healthcare barriers due to communication difficulties, sensory sensitivities, and a lack of specialized training among providers, while systemic issues like inadequate infrastructure and limited support further hinder access. This study aims to identify barriers to quality and timely healthcare for individuals with autism in four European countries.

Methods Two subsamples were created: one of family members (138 participants) and another of healthcare workers (237 participants). Data were collected online from North Macedonia, Serbia, Cyprus, and Spain.

Results Significant differences were found between countries regarding healthcare barriers. Participants from Serbia and North Macedonia reported greater issues with system integration, limited consultation time, and insufficient support compared with those from Cyprus, while Spanish participants rated phone appointment scheduling as a more significant barrier than those from Cyprus. About one-third of healthcare workers felt comfortable treating patients with autism, with Cypriot participants being more at ease with communication and those from North Macedonia being more comfortable administering and monitoring medications. Spanish healthcare workers perceived communication difficulties, sensory processing issues, and anxiety in patients with autism as greater obstacles than did those from other countries, while they also rated insufficient training as a more significant barrier than those from Cyprus.

Conclusion This study highlights the need for autism-specific accommodations, comprehensive training for healthcare professionals, and policy reforms to improve healthcare accessibility and outcomes for individuals with autism.

Keywords: autism spectrum disorder; healthcare services; training

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by persistent deficits in initiating and maintaining communication and reciprocal social interactions, as well as restricted, stereotypical, and repetitive patterns of behavior, interests, and activities [1]. The prevalence of ASD is estimated to be around 1% [2].

Individuals with ASD are two to three times more likely to suffer from neurological and rheumatological diseases, as well as liver, kidney, and gastrointestinal disorders compared to the general population. Research indicates that diseases affecting almost all organs and organ systems are more prevalent in patients with ASD than in those without autism [3]. Furthermore, more than half of individuals with autism have at least one psychiatric diagnosis. The most common comorbid psychiatric conditions include attention-deficit/hyperactivity disorder, mood disorders, and anxiety, while schizophrenia spectrum disorders are also relatively frequent [4]. Comorbid conditions are associated with poorer outcomes. These patients require specific clinical management, good coordination between different healthcare services, and additional financial resources [5].

Unfortunately, patients with ASD face numerous barriers to accessing healthcare services. Some of these barriers are directly related to the characteristics of autism, such as difficulties in communication and sensory hypersensitivity. A lack of knowledge and specialized training for working with ASD patients are examples of barriers associated with healthcare providers. Certain obstacles are deeply rooted within the healthcare system itself, including the unavailability of support for both ASD patients and healthcare professionals, inadequate infrastructure, time constraints, and system inflexibility. Additionally, caregivers can sometimes create further barriers due to their preference for alternative treatment methods and lack of cooperation with healthcare services [6].

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Research on the accessibility of healthcare systems and potential obstacles to obtaining healthcare services analyzes the opinions and perspectives of individuals with autism, their parents or caregivers, and healthcare professionals. As expected, different groups of respondents offer diverse viewpoints regarding the barriers to accessing quality healthcare. Furthermore, various European countries have different practices and past experiences in providing specialized support for ASD patients. Therefore, the aim of this study is to identify the barriers to accessing quality and timely healthcare for individuals with autism in four European countries.

METHODS

Sample

Two subsamples were created in order to assess the current situation regarding the provision of healthcare services to people with autism. The first subsample included family members of people with autism, and the second consisted of healthcare workers. Both were convenience samples.

We contacted potential participants from the first subsample through national and/or local organizations that provide support to people with autism from North Macedonia, Serbia, Cyprus, and Spain.

The first subsample included 138 participants aged 17–77 years [mean (M) = 45.32; standard deviation (SD) = 11.12]. Most participants were female (108, or 78.3%), while slightly over one-fifth were male (30, or 21.7%). The majority of the first subsample were mothers of people with autism (101, or 73.2%), while there were significantly fewer fathers (22 or 15.9%). The remaining 15 (10.9%) participants were other family members of people with autism (siblings, grandmothers, and aunts) (Table 1).

Family members with autism were 3–53 years of age (M = 15.81; SD = 10.56). Most individuals with autism (55, or 39.9%) were at the second level of support needs. Thirty-seven (26.8%) individuals with autism were at the lowest level of support needs, while the remaining 46 (33.3%) were at the highest level (Table 2).

The value of Fisher's exact test (13.435; $p < 0.05$) indicates differences in the level of support needs. In Spain, participants at the highest level of support needs were predominant. In North Macedonia and Serbia, most individuals with autism from families in the first subsample were at the medium level of support needs, followed by those at the highest level, while the smallest percentage comprised individuals with high-functioning autism (21.6% and 16% for North Macedonia and Serbia, respectively). In the subsample of participants from Cyprus, the number of participants at the lowest and medium levels of support needs was equal, while 16.7% were at the highest level.

The second subsample, also a convenience sample, included 237 participants aged 20–68 years (M = 40.54; SD = 10.41). Most participants were female (186, or 78.5%), while about one-fifth of participants (47, or 19.8%) were male. Four participants (1.7%) chose not to declare their gender. Half of the participants (119 or 50.2%) were nurses and technicians. The sample also included 12 (5.1%) general practitioners, 31 (13%) resident physicians, 47 (19.8%) specialists, six (2.5%) dentists, and 22 (9.3%) other healthcare professionals and associates, most of whom were physical therapists (n = 8) and radiology technicians or radiographers (n = 6).

Resident physicians and specialists covered very different medical fields. The majority were pediatricians (n = 20). Some were subspecialists in pediatric immunology, developmental pediatrics, neuropediatrics, or pediatric gastroenterology. They were followed by specialists in family medicine (n = 6) and psychiatry (n = 5). There were three specialists in each of the following fields: transfusion medicine, surgery, and nuclear medicine. Numerous other medical branches were represented by one or two participants each (dermatology, ophthalmology, neurology, hematology, anesthesiology, cardiology, gynecology, pathology, internal medicine, radiology, etc.). Slightly over one-third of the participants (84 or 35.4%) graduated from university. They were followed by participants who completed high school (50, or 21.1%) and a vocational college (42, or 17.7%). Forty participants (16.9%) had a master's degree, while 21 (8.9%) had a PhD (Table 3). Given the wide age range, it is not surprising that the participants also differed substantially in work experience, which ranged 1–48 years (M = 15.55; SD = 10.58).

Most participants (83, or 35%) acquired only basic knowledge about autism during their undergraduate studies. Slightly over one-fourth of the participants (64, or 27%) had never received any education on autism. About one-fifth of participants (47, or 19.8%) completed some form

Table 1. Sample characteristics – family members (N = 138)

Country	N	Age M (SD)	Sex		Family member		
			female	male	mother	father	other
			N	N	N	N	N
North Macedonia	37	42.68 (6.79)	31	6	27	5	5
Serbia	25	52.4 (12.28)	22	3	20	3	2
Cyprus	24	43.42 (10.39)	17	7	15	6	3
Spain	52	44.67 (12.17)	38	14	39	8	5

Table 2. Family members with autism spectrum disorder (N = 138)

Country	N	Age M (SD)	Level of support		
			I (N)	II (N)	III (N)
North Macedonia	37	12.14 (5.46)	8	19	10
Serbia	25	22.48 (9.56)	4	13	8
Cyprus	24	14.29 (9.93)	10	10	4
Spain	52	15.92 (10.56)	15	13	24

Table 3. Age and education level of healthcare workers (N = 237)

Country	N	Age M (SD)	Education level		
			high school	college/ university	master's/ PhD
North Macedonia	49	39.88 (8.28)	5	36	8
Serbia	102	40.33 (10.77)	45	45	12
Cyprus	26	36.69 (7.72)	0	9	17
Spain	60	42.73 (11.91)	0	36	24

Table 4. Obstacles to accessing healthcare services – assessment by family members of people with autism (percentage values)

Item	Not an obstacle	Not an important obstacle	Neutral	Somewhat of an obstacle	A very important obstacle
Communication difficulties experienced by people with ASD.	6.5	8	3.6	33.3	48.6
Difficulties in sensory processing.	9.4	11.6	8.7	30.4	39.9
Anxiety, fear, and refusal to cooperate during the examination.	8	9.4	8.7	26.1	47.8
Self-harm and aggression.	31.9	8	11.6	18.8	29.7
Insufficient training of healthcare workers in working with individuals with autism.	5.8	8.7	5.8	27.5	52.2
Inflexibility of healthcare staff in approaching a person with autism.	8	8.7	11.6	27.5	44.2
Insufficient motivation of healthcare workers to help a person with autism.	7.2	13	9.4	26.8	43.5
Neglecting the attitudes and suggestions of persons with autism and/or their family members.	9.4	10.9	9.4	32.6	37.7
The doctor has no insight into the reports of other professionals because the system is not integrated.	8	8	13	23.2	47.8
No extra time to work with an autistic patient.	5.1	8	7.2	20.3	59.4
Adequate incentives are not provided for working with an autistic patient.	3.6	7.2	10.9	24.6	53.6
The family cannot cover all health needs of a person with ASD with their existing medical insurance.	8.7	9.4	8	15.9	58
A person with ASD or their family member has difficulty with the appointment system online or over the phone.	6.5	12.3	14.5	26.8	39.9

Table 5. Level of comfort in working with people with autism

How comfortable do you feel	Completely uncomfortable		Uncomfortable		Neutral		Comfortable		Completely comfortable	
	N	%	N	%	N	%	N	%	N	%
Examining a patient	23	9.7	56	23.6	68	28.7	73	30.8	17	7.2
Communicating with a patient	25	10.5	68	28.7	59	24.9	67	28.3	18	7.6
Discussing the medical plan	27	11.4	60	25.3	80	33.8	57	24.1	13	5.5
Using pictures to communicate	26	11	46	19.4	93	39.2	54	22.8	18	7.6
Inquiring about communication needs	16	6.8	61	25.7	84	35.4	59	24.9	17	7.2
Administering medications and monitoring effects	28	11.8	57	24.1	82	34.6	55	23.2	15	6.3
Cooperating with parents	17	7.2	40	16.9	61	25.7	76	32.1	43	18.1
Cooperating with other professionals	15	6.3	37	15.6	61	25.7	83	35	41	17.3
Finding adequate information about autism	13	5.5	41	17.3	75	31.6	79	33.3	29	12.2

of informal training. Significantly fewer participants (26, or 11%) acquired basic knowledge about autism through their postgraduate studies. Only 17 participants believed that they had thorough knowledge about autism acquired either during undergraduate studies (five, or 2.1%) or postgraduate studies (12, or 5.1%).

Healthcare workers most often claimed that they rarely had patients with autism (103, or 43%), while 72 (30.4%) occasionally had patients with ASD. Almost one-fifth of the participants (46, or 19.4%) reported never having had a patient with autism, while 16 (6.8%) were in frequent professional contact with patients with autism.

Assessment instrument

This research, part of the European project HeAlth: Improving access to healthcare services for people with autism, involved creating the Barriers in Healthcare survey for healthcare workers and family members of individuals with autism. The survey, developed based on a systematic review of national and international evidence, was analyzed by a consulting group, including two people with autism and their family members. It was translated into national languages and distributed to participants. The survey had two parts: one collected participant demographics, and the other assessed healthcare barriers using a five-point Likert scale. Participation was voluntary, with informed consent provided by all participants.

Data analysis

The results are presented in absolute numbers and percentages. Since the conditions for parametric statistical techniques were not met (violations of the assumptions of normality and homogeneity of variance, along with unequal subsample sizes), differences in scores of participants from different countries were examined by the Kruskal–Wallis test. In cases where statistically significant differences were found, we applied a series of Mann–Whitney U tests to determine more precisely which groups differed. The Bonferroni correction was applied in each case to avoid type I errors.

RESULTS

We will first present the results obtained from the subsample of family members of persons with autism and then the results from healthcare workers.

Family members of persons with autism

The overall distribution of family members' ratings of barriers to healthcare access is presented in Table 4. The Kruskal–Wallis test revealed statistically significant differences between family members of people with autism in different countries on four items.

The statement that 'the doctor has no insight into the reports of other professionals because the system is not integrated' was rated differently by participants from different countries – $\chi^2(3, N = 138) = 9.374$, $p = 0.025$. Participants from Serbia and North Macedonia had a higher median score (Md = 5) than participants from Spain (Md = 4) and Cyprus (Md = 3). We conducted post hoc comparisons using a series of Mann–Whitney U tests, with Bonferroni adjustment of the alpha value, to determine which groups differed significantly. The results showed that participants from North Macedonia significantly differed from the participants from Cyprus ($p = 0.008$, $r = 0.23$), while the difference between participants from Serbia and those from Cyprus was on the borderline of statistical significance ($p = 0.012$, $r = 0.21$). According to Cohen's criteria, the effect size approaches medium values ($r > 0.2$).

The Kruskal–Wallis test also showed differences in ratings of the statement that doctors have no extra time to work with autistic patients – χ^2 (3, N = 138) = 8.857, $p = 0.031$. Participants from Cyprus had a lower median score (Md = 4) than all other participants (Md = 5). However, post hoc comparisons using the Mann–Whitney U test, with Bonferroni adjustment of the alpha value, found statistically significant differences only between participants from Serbia and those from Cyprus, with an effect size between small and medium values ($p = 0.008$, $r = 0.22$).

Participants from North Macedonia, Serbia, and Spain agreed with the statement that doctors are not provided with adequate incentives for working with autistic patients (Md = 5) much more than participants from Cyprus (Md = 4) – χ^2 (3, N = 138) = 14.026, $p = 0.003$. Post hoc comparisons using the Mann–Whitney U test, with Bonferroni adjustment of the alpha value, found statistically significant differences between participants from North Macedonia and participants from Cyprus ($p < 0.001$, $r = 0.31$), as well as between participants from Serbia and those from Cyprus ($p = 0.004$, $r = 0.25$), with a medium effect size.

Significant differences were also found in ratings of the statement that making an appointment over the phone is an obstacle to healthcare system accessibility – χ^2 (3, N = 138) = 8.489, $p = 0.037$. Participants from Cyprus had the lowest median (Md = 3), followed by participants from North Macedonia and Serbia (Md = 4), and, finally, participants from Spain (Md = 4.5). After conducting the Mann–Whitney U tests, with Bonferroni adjustment, the only significant difference was found between participants from Cyprus and those from Spain ($p = 0.007$, $r = 0.23$), with an effect size that, according to Cohen's criteria, falls between small and medium values.

Healthcare workers

The results shown in Table 5 indicate that about one-third of the participants felt comfortable or completely comfortable when examining patients with autism and communicating with them, discussing a medical plan, using pictures to communicate, determining communication needs, administering medications and monitoring their effects. About half of the healthcare workers felt comfortable or completely comfortable cooperating with parents and other healthcare professionals, as well as finding adequate information about autism. An initial analysis using the Kruskal–Wallis test indicated possible differences in the ratings of three items by participants from different countries. However, post hoc comparisons using the Mann–Whitney U test found statistically significant differences only in individual cases on two items. Participants from Cyprus were more comfortable communicating with autistic patients than participants from Spain ($p = 0.004$; $r = 0.19$), while participants from North Macedonia felt more comfortable administering medications to autistic patients and monitoring their effects ($p = 0.002$; $r = 0.2$). In both cases, the effect size falls between small and medium values.

Healthcare workers' ratings of barriers to healthcare access are presented in Table 6. Healthcare workers' assessments of the obstacles faced by people with autism in the healthcare system revealed significant differences between countries on four items. Three items refer to obstacles related to the symptoms of autism, while one item is related to healthcare workers. Since the median values were almost identical, in examining potential differences, we relied on mean ranks and post hoc comparisons using the Mann–Whitney U test, with Bonferroni adjustment of the alpha value. The mean rank values were the highest among participants from Spain on all four items, while they were relatively uniform among participants from other countries.

Participants from different countries differed in their assessments of the extent to which communication difficulties experienced by people with ASD (e.g., identifying and communicating symptoms) represented an obstacle to accessing the healthcare system – χ^2 (3, N = 237) = 15.267, $p = 0.002$. The Mann–Whitney U test showed that participants from Spain rated this item significantly higher than participants from Serbia ($p < 0.001$, $r = 0.31$) and those from Cyprus ($p = 0.002$, $r = 0.26$), with a medium effect size.

Participants from different countries also differed in their assessments of the extent to which sensory processing difficulties experienced by people with autism posed an obstacle to accessing the healthcare system – χ^2 (3, N = 237) = 11.277, $p = 0.01$. Participants from Spain rated this item significantly higher than participants from North Macedonia ($p = 0.013$, $r = 0.21$), Serbia ($p = 0.002$, $r = 0.26$), and Cyprus ($p = 0.012$, $r = 0.21$). According to Cohen's criteria, the effect size is between small and medium.

Statistically significant differences between participants from different countries were also found regarding the extent to which anxiety, fear, and refusal to cooperate during

Table 6. Obstacles to accessing healthcare services – assessment by healthcare workers (percentage values)

Item	Not an obstacle	Not an important obstacle	Neutral	Somewhat of an obstacle	A very important obstacle
Communication difficulties experienced by people with ASD.	4.2	16.5	9.3	41.8	28
Difficulties in sensory processing.	4.2	13.9	12.7	40.9	28.3
Anxiety, fear, and refusal to cooperate during the examination.	4.2	12.2	10.5	32.1	40.9
Self-harm and aggression.	4.2	13.9	16	27.8	38
Insufficient training of healthcare workers in working with individuals with autism.	4.6	11.8	12.2	30.4	40.9
Inflexibility of healthcare staff in approaching a person with autism.	6.3	12.2	16.9	34.2	30.4
Insufficient motivation of healthcare workers to help a person with autism.	9.3	11.8	22.4	32.1	25
Neglecting the attitudes and suggestions of persons with autism and/or their family members.	7.6	10.1	21.9	32.5	37.9
The doctor has no insight into the reports of other professionals because the system is not integrated.	8	13.5	18.1	29.1	31.2
No extra time to work with an autistic patient.	7.6	9.3	15.2	30	38
Adequate incentives are not provided for working with an autistic patient.	7.2	12.7	19.8	28.3	32.1
The family cannot cover all health needs of a person with ASD with their existing medical insurance.	5.9	10.5	18.6	25.7	39.2
A person with ASD or their family member has difficulty with the appointment system online or over the phone.	9.3	13.1	17.3	33.3	27

the examination of people with autism posed an obstacle to accessing the healthcare system – χ^2 (3, N = 237) = 9.445, p = 0.024. The Mann–Whitney U test showed that participants from Spain rated this item higher than participants from Serbia (p = 0.009, r = 0.22) and those from Cyprus (p = 0.005, r = 0.24), with the effect size approaching medium values.

The Kruskal–Wallis test indicated differences between participants from different countries regarding the opinion that insufficient training of healthcare workers represented an obstacle to people with autism – χ^2 (3, N = 237) = 7.858, p = 0.049. However, post hoc comparisons found statistically significant differences only between participants from Spain and those from Cyprus (p = 0.007, r = 0.23).

DISCUSSION

Family members and medical professionals report notable differences in perceived healthcare barriers for people with autism, highlighting the systemic and cultural impacts across nations. The findings align with previous studies that show significant obstacles in healthcare access, particularly due to the challenges of navigating healthcare systems and the inadequacy of traditional communication methods for autistic patients.

This study supports findings from other research, which identified poor communication between patients and doctors as a source of misinterpretations, incorrect diagnoses, and frustration [7]. The study confirms that communication remains a significant barrier in many nations, particularly when providers are not trained in autism-specific communication. Additionally, previous studies have highlighted sensory problems in healthcare settings, and this study demonstrates the continued unmet need for sensory adjustments worldwide [8, 9].

Recent evidence indicates that autistic adults experience multiple barriers to accessing healthcare, including systemic and organizational obstacles that contribute to unmet needs and difficulties in navigating services, underscoring disparities in care even within public health systems [10].

Research highlights the importance of support networks in improving healthcare access. Mazurek et al. [11] found that individuals with strong family and community support face fewer healthcare barriers, which aligns with this study. It shows how these networks vary between nations, with better results in countries with stronger autism advocacy and support.

The effectiveness of current autism training programs is also a key issue. Previous studies, including a recent systematic review, have shown that many healthcare professionals feel insufficiently prepared to care for autistic patients, largely due to limited autism-specific training during medical education and clinical practice [12]. Building on research by Crane et al. [13], this study emphasizes that current training programs often address general disabilities rather than autism-specific needs. It underscores the necessity for training healthcare providers to address challenges specific to autism, such as sensory sensitivities and alternative communication methods, to improve care for autistic patients.

Prior studies have emphasized the need for structured, comprehensive autism training within healthcare programs. This study builds on those recommendations by highlighting the specific training needs identified by healthcare workers, such as communication techniques and sensory accommodations. It stresses the global need for standardized autism training focused on practical skills rather than just theoretical knowledge, supporting findings from other studies [14]. Research on cultural competence training further suggests that such training may improve professionals' preparedness to work with diverse populations and some patient-reported aspects of care, although evidence of direct effects on health outcomes remains inconclusive [15, 16].

The analysis revealed several country-specific differences in the perceived barriers to healthcare access for autistic individuals, although the pattern differed between family members and healthcare workers. Among family members, participants from Serbia and North Macedonia reported higher ratings of several organizational barriers, particularly limited integration between healthcare professionals, insufficient time for physicians, and insufficient incentives for working with autistic patients, whereas participants from Cyprus generally reported lower ratings of these barriers. In contrast, healthcare workers from Spain reported higher ratings of autism-specific barriers, including communication and sensory difficulties and anxiety or refusal to cooperate during examinations. These differences may reflect variations in the organization and integration of healthcare services, the availability of specialized autism services, professional education and training, exposure to autistic patients, and cultural expectations regarding healthcare, although these factors were not directly assessed in the present study. The higher ratings reported by Spanish healthcare workers may, for example, reflect greater recognition of autism-specific challenges rather than necessarily greater barriers in the healthcare system itself. However, this interpretation should be considered hypothetical. The differing patterns reported by family members and healthcare workers further suggest that healthcare accessibility is multidimensional and may be influenced by both system-level characteristics and the recognition of autism-specific needs. Future comparative research should therefore examine country-level differences in service organization, autism-specific training, national policies, and the availability of specialized services.

These findings have several practical implications for improving healthcare accessibility for autistic individuals. First, healthcare professionals should receive standardized autism-specific training that includes practical skills in communication, recognition of sensory needs, and adaptation of healthcare procedures to individual needs. Second, healthcare settings should consider sensory-friendly adaptations, such as reducing unnecessary sensory stimuli and providing appropriate communication supports. Third, healthcare services should consider more flexible appointment procedures and longer consultation times when needed, as well as better integration and exchange of information between professionals. Together, these measures could contribute to more accessible and responsive healthcare for autistic individuals.

Limitations

There are several limitations that should be considered when interpreting the findings. First, the use of convenience sampling and relatively small samples within individual countries limits the generalizability of the results and may have affected the comparability of the country groups. Differences in sample composition across countries, including participants' professional or family roles and their experience with autistic individuals, may also have contributed to the observed cross-country differences. Second, the study relied on self-reported data, which may be influenced by participants' individual experiences, expectations, and perceptions of autism and healthcare services. Third, the study did not systematically assess potentially important country-level factors, such as differences in healthcare organization, availability of specialized autism services, autism-specific professional training, national policies, or clinical guidelines. In addition, differences in the way autism is perceived, diagnosed, and supported across countries may have influenced participants' responses and may partly account for the observed cross-country differences. These factors should therefore be considered when interpreting the findings, particularly when making international comparisons. Future comparative studies should include more representative and comparable samples and incorporate systematic measures of healthcare systems, professional training, and national autism policies.

CONCLUSION

This study highlights the barriers autistic individuals face in accessing healthcare and identifies differences in the perceived barriers across the four countries included in the study. The findings indicate the importance of addressing autism-specific needs within healthcare services, particularly in relation to communication, sensory needs, healthcare professionals' training, and the organization and integration of services. Practical measures may include standardized autism-specific training for healthcare professionals, sensory-friendly adaptations of healthcare environments, appropriate communication supports, flexible appointment procedures, and improved coordination and information sharing between professionals. These measures may contribute to more accessible and responsive healthcare for autistic individuals. Further comparative research is needed to examine how healthcare organization, professional training, national policies, and the availability of specialized autism services influence healthcare accessibility across countries.

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All authors meet the conditions for authorship. All authors contributed to the conception and design, the analysis, and interpretation of the data. All authors have intellectually conceived of and critically reviewed the manuscript and agree with its final version.

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Препреке у приступу системима здравствене заштите са којима се суочавају особе са аутизмом – студија у четири европске земље

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САЖЕТАК

Увод/Циљ Особе са аутизмом суочавају се са значајним препрекама у приступу здравственој заштити због тешкоћа у комуникацији, сензорне осетљивости и недостатка специјализоване обуке пружалаца здравствених услуга, док системски проблеми, као што су неадекватна инфраструктура и ограничена подршка, додатно отежавају приступ. Циљ ове студије је да идентификује препреке за квалитетну и правовремену здравствену заштиту особа са аутизмом у четири европске земље.

Методе Формирана су два подузорка: један од чланова породица (138 учесника) и други од здравствених радника (237 учесника). Подаци су прикупљени онлајн из Северне Македоније, Србије, Кипра и Шпаније.

Резултати Утврђене су значајне разлике између земаља у вези са препрекама у здравственој заштити. Учесници из Србије и Северне Македоније пријавили су веће проблеме са увезаношћу услуга унутар система, недостатком времена лекара и недовољном подршком у поређењу са учесницима са Кипра, док су учесници из Шпаније заказивање термина телефоном оценили као већу препреку него учесници са Кипра. Отприлике трећина здравствених радника осећала се комфорно приликом лечења пацијената са аутизмом, при чему су се учесници са Кипра осећали комфорније у комуникацији, а учесници из Северне Македоније у примени лекова и праћењу терапије. Здравствени радници из Шпаније сматрали су тешкоће у комуникацији, проблеме са сензорним процесирањем и анксиозност код пацијената са аутизмом већим препрекама него здравствени радници из других земаља, а недостатак обуке оценили су као већу препреку него учесници са Кипра.

Закључак Ова студија указује на потребу за прилагођавањем здравствене заштите специфичним потребама особа са аутизмом, свеобухватним обукама здравствених радника и реформама здравствене политике како би се побољшали приступ здравственој заштити и здравствени исходи особа са аутизмом.

Кључне речи: поремећај из спектра аутизма; здравствене услуге; обука

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

An atypical groin mass in a middle-aged woman – inguinal leiomyoma as a rare clinical entity

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SUMMARY
Introduction Leiomyomas are benign smooth muscle tumors that rarely occur outside the uterus. Inguinal canal leiomyomas are particularly uncommon and may resemble hernias, endometriosis, lymphadenopathy, or other soft tissue lesions, making preoperative diagnosis difficult.
Case outline We report the case of a 47-year-old woman with a painless right inguinal mass, first noticed approximately 1.5 years before surgery and progressively enlarged without associated systemic symptoms. Examination revealed a mobile, well-defined, non-tender lesion. Ultrasound showed a heterogeneous, predominantly hypoechoic, vascular mass. Computed tomography demonstrated a hypodense lesion with heterogeneous enhancement and preserved surrounding fat planes. Magnetic resonance imaging revealed a well-circumscribed, partially cystic mass without infiltration or fatty or hemorrhagic components. Its proximity to the round ligament and arterial supply from a branch of the inferior epigastric artery raised the possibility of leiomyoma, endometriosis, or another benign soft tissue tumor. Due to progressive growth and uncertain etiology, the mass was surgically removed in late 2024. Gross examination showed an encapsulated, whitish, elastic lesion. Histology demonstrated a mesenchymal proliferation without atypia or necrosis. Positive staining for α-smooth muscle actin, caldesmon, desmin, estrogen receptors, and progesterone receptors, together with a Ki-67 index below 1%, established the diagnosis of leiomyoma. Two postoperative abdominal ultrasound examinations, including both inguinal regions, showed no evidence of local recurrence. The patient remains asymptomatic.
Conclusion Inguinal canal leiomyoma should be considered in the differential diagnosis of a slowly growing groin mass. Imaging defines its extent and relationship to adjacent structures, while histopathology and immunohistochemistry establish the definitive diagnosis and guide treatment.
Keywords: leiomyoma; inguinal canal; groin mass; differential diagnosis; case report

INTRODUCTION

Leiomyomas are among the most frequent benign tumors of the female genital tract, affecting nearly one in three women of reproductive age [1]. While they usually develop within the uterine myometrium and may lead to abnormal bleeding, pelvic pain, or infertility, their extragenital occurrence is uncommon. Rarely, they have been described in sites such as the broad ligament, the round ligament, and, in exceptional cases, the inguinal canal [2].

The diagnostic challenge of inguinal leiomyomas stems from their ability to mimic far more prevalent conditions, including inguinal hernia, lymphadenopathy, cyst of the canal of Nuck, or endometriosis [3]. Because of their rarity and nonspecific presentation, reaching a preoperative diagnosis is often difficult. Multimodal imaging – most notably ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) – can help refine the differential diagnosis, yet histopathological and immunohistochemical evaluation remain essential for definitive confirmation [4].

Awareness of this entity is crucial, since overlooking it may lead to misdiagnosis and even unnecessary oncological treatment. This report describes the case of a 47-year-old woman with an inguinal canal leiomyoma, emphasizing the radiological findings, surgical outcome, and histopathological correlation, and discussing the key considerations that distinguish this rare lesion from other groin masses.

CASE REPORT

A 47-year-old woman presented with a progressively enlarging, painless mass in the right inguinal region. She denied systemic symptoms such as weight loss, fever, or night sweats. On physical examination, the lesion was mobile, well-circumscribed, and non-tender, with no signs of overlying skin changes.

Laboratory analyses revealed normal hematological and biochemical parameters. Tumor markers, including carcinoembryonic antigen, α-fetoprotein, human epididymis protein 4, and cancer antigen 15-3, were within reference ranges.

US examination demonstrated a heterogeneous, predominantly hypoechoic, well-circumscribed mass with internal vascularity (Figures 1 and 2). CT imaging revealed a hypodense lesion with heterogeneous post-contrast enhancement and preserved fat planes relative to surrounding musculature and iliac vessels (Figures 3 and 4).

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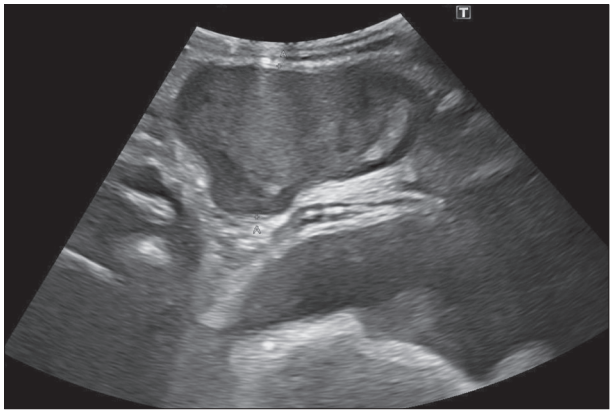


Figure 1. Lobulated soft tissue mass in the right inguinal region with heterogeneous structure – convex probe

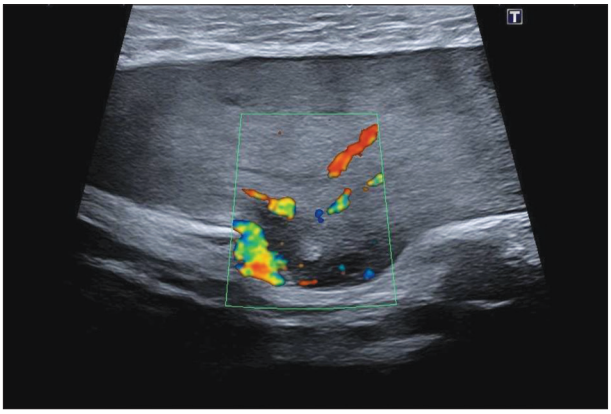


Figure 2. Well-defined hypoechoic lesion, moderately vascularized in Doppler mode – imaged with a linear probe

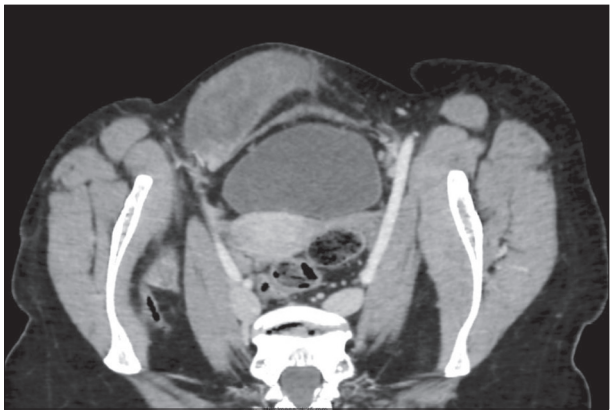


Figure 3. An axial CT image obtained after intravenous contrast administration shows a sharply defined, hyperdense lesion in the right inguinal region, exhibiting moderate post-contrast enhancement

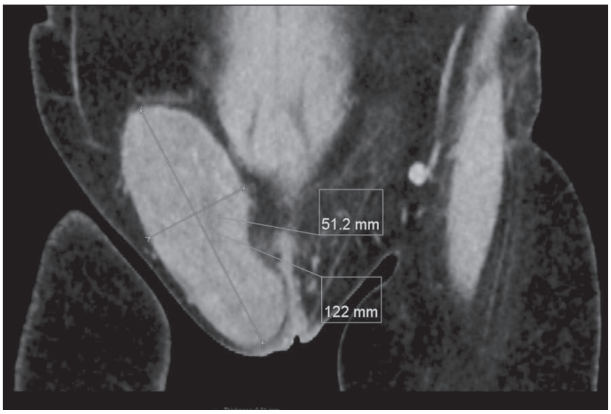


Figure 4. A coronal CT image obtained after intravenous contrast administration shows a sharply contoured heterodense lesion, measuring 120 × 50 mm, in the right inguinal region, with extension into the perineum

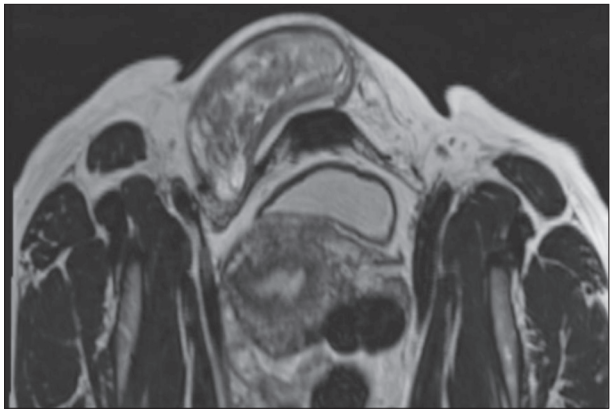


Figure 5. An axial T2-weighted turbo spin-echo sequence demonstrates a subcutaneous, right inguinal, oval-shaped, sharply contoured lesion with heterogeneous signal intensity and areas of cystic degeneration

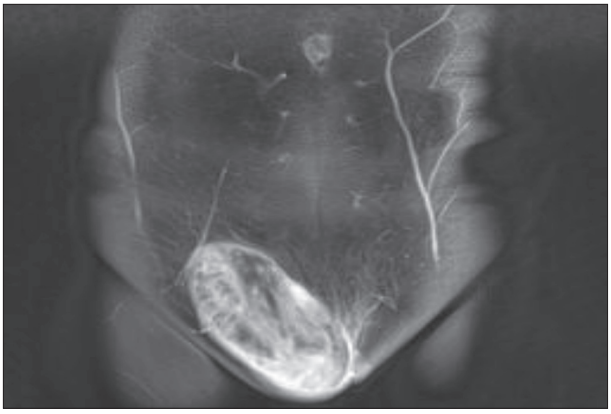


Figure 6. A coronal T1-weighted volumetric interpolated breath-hold examination sequence with fat suppression demonstrates an oval, sharply contoured lesion with heterogeneous signal intensity and solid and cystic components in the right inguinal area, showing moderate post-contrast enhancement of the solid component; the lesion measures 120 × 45 mm in the coronal plane

MRI confirmed a well-encapsulated, partially cystic mass, hyperintense on T2-weighted sequences, without evidence of infiltration or intraperitoneal extension (Figures 5 and 6).

Summary across modalities: across all imaging modalities (US, CT, and MRI), the lesion in the right inguinal region consistently appeared as a well-circumscribed, encapsulated, heterogeneous mass with partially cystic components, showing internal vascularity and heterogeneous post-contrast enhancement. Importantly, fat planes separating the lesion from adjacent iliac vessels and surrounding musculature were preserved, and there were no imaging signs of invasion or intraperitoneal extension. The imaging constellation favored a benign soft tissue tumor, with leiomyoma considered among the leading differential diagnoses, along with other benign inguinal lesions such as a canal of Nuck cyst or endometriosis.

Surgical management: Following preparation of the surgical field, a right oblique inguinal incision was made. After careful dissection of the inguinal canal, the mass was completely excised. Gross examination revealed an encapsulated lesion measuring 100 × 65 × 40 mm, whitish in color, with a smooth, glistening surface and elastic consistency.

Histopathology: microscopic evaluation revealed a mesenchymal proliferation of moderate cellularity composed of uniform spindle cells with eosinophilic cytoplasm and elongated nuclei with blunt ends. No atypia, necrosis, or significant mitotic activity was identified. The stroma showed focal hyalinization and mild myxoid changes. Immunohistochemistry demonstrated positivity for α-smooth muscle actin (α-SMA), caldesmon, desmin, estrogen receptors, and progesterone receptors, with negativity for SOX10 and S100. Ki-67 was expressed in < 1% of tumor cells. The final diagnosis was leiomyoma.

DISCUSSION

Extragenital leiomyomas are exceedingly uncommon, and when located in the inguinal canal, they pose a considerable diagnostic challenge. Most of the reported cases occur in women of reproductive age, likely due to the hormonal responsiveness of these tumors [5]. The proximity to the round ligament and the expression of estrogen and progesterone receptors in our case support the role of hormonal influence.

Clinically, inguinal leiomyomas are frequently mistaken for more common pathologies such as hernias, lymphadenopathy, canal of Nuck cysts, or endometriosis [6]. Imaging plays a pivotal role in narrowing the differential diagnosis. US often demonstrates a solid, vascularized mass, while CT and MRI can provide better definition of lesion margins and the relationship with adjacent structures [7]. However, as in our case, imaging cannot always reliably distinguish between leiomyomas and other benign or malignant soft tissue tumors.

Histopathological examination remains the gold standard for diagnosis. Typical features include uniform spindle cells arranged in fascicles, absence of significant atypia, and low mitotic activity. Immunohistochemistry is invaluable in confirming smooth muscle differentiation (α-SMA, desmin, and caldesmon positivity) and excluding other spindle cell neoplasms such as schwannomas or sarcomas [8].

Surgical excision is the treatment of choice, with an excellent prognosis and minimal recurrence risk when margins are free. Reported recurrence is exceedingly rare, supporting the benign nature of this entity [9].

Notably, a comprehensive review of the literature identified no reported cases of round ligament leiomyoma recurrence over the preceding 25 years, reinforcing the favorable long-term outcome following complete surgical excision [10]. Consistent with these findings, our patient underwent two postoperative ultrasound examinations, including targeted examination of both inguinal regions, which showed no evidence of local recurrence. The patient remains asymptomatic and in good general condition.

Therefore, accurate recognition of this rare lesion can prevent unnecessary radical or oncological procedures.

Heightened awareness of this entity among radiologists, gynecologists, and surgeons is essential to prevent misdiagnosis and unnecessary oncological interventions, and this entity should remain an integral consideration in the differential diagnosis of inguinal masses in women.

Ethics: All procedures performed were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments and comparable ethical standards. Written consent to publish all material presented was obtained from the patient.

Conflict of interest: None declared.

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Атипична маса у препонској регији код жене средњих година – ингвинални лејомиом као редак клинички ентитет

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САЖЕТАК

Увод Лејомиоми су бенигни тумори глатке мускулатуре који се најчешће јављају у утерусу, док је њихова екстрагенитална локализација изузетно ретка, нарочито у ингвиналном каналу. Због клиничке сличности са хернијом, ендометриозом, лимфаденопатијом и мекоткивним лезијама, представљају дијагностички изазов. Препознавање ове неуобичајене локализације важно је за избегавање погрешне дијагнозе и неадекватног лечења.

Приказ болесника Приказујемо 47-годишњу болесницу са безболном масом у десној ингвиналној регији, први пут уоченом приближно годину и по дана пре операције, која је постепено расла без системских симптома. Клиничким прегледом идентификована је покретна, јасно ограничена и индолентна формација. Ултразвуком је приказана хетероехогена, доминантно хипоехогена, васкуларизована маса. Компјутеризована томографија показала је хиподензну промену са нехомогеним постконтрастним појачањем и очуваним околним масним плановима. Магнетном резонанцом уочена је добро ограничена, делимично цистична маса, без инфилтрације околних структура, масног или хеморагичног садржаја. Због блиског односа са округлим лигаментом материце и васкуларизације из гране доње епигастричне артерије, разматрани су лејомиом, ендометриоза и други бенигни мекоткивни тумори. Услед прогресивног раста и нејасне етиологије, промена је хируршки одстрањена крајем 2024. године. Макроскопски је била инкапсулирана, беличаста, еластичне конзистенције. Патохистолошка анализа показала је мезенхималну пролиферацију без атипије и некрозе. Позитивност на *α-SMA*, калдесмон, дезмин, естрогенске и прогестеронске рецепторе, уз *Ki*-67 испод 1%, потврдила је дијагнозу лејомиома. Два постоперативна ултразвучна прегледа абдомена, укључујући обе ингвиналне регије, нису показала знаке локалног рецидива. Болесница је без тегоба.

Закључак Случај истиче значај интегрисаног клиничког, радиолошког и патохистолошког приступа у процени атипично локализованих тумора меких ткива. Правовремено препознавање бенигне природе промене омогућава одговарајуће лечење и избегавање непотребних онколошких процедура код тумора који клинички имитирају малигне или инфламаторне ентитете.

Кључне речи: лејомиом; ингвинални канал; препонска маса; диференцијална дијагноза; приказ случаја

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Primary extragonadal seminoma of the urinary bladder presenting with spontaneous hemoperitoneum and hemorrhagic shock – a rare case report

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SUMMARY

Introduction Extragonadal seminomas are rare germ cell tumors that most often occur in the mediastinum and retroperitoneum. Primary localization in the urinary bladder is extremely rare and represents a diagnostic challenge. Acute presentation in the form of intra-abdominal bleeding and hemorrhagic shock has so far been extremely rarely described.

Case report We present the case of a 36-year-old man who was admitted with severe abdominal pain and clinical signs of an acute abdomen and hemorrhagic shock. Laboratory analyses showed anemia (hemoglobin 58 g/L) and a pronounced inflammatory reaction (C-reactive protein 314.8 mg/L). Ultrasound examination of the abdomen revealed a large amount of free intraperitoneal fluid, while native radiography of the abdomen showed several hydroaeric levels without signs of pneumoperitoneum. Due to hemodynamic instability and signs of peritoneal irritation, an urgent exploratory laparotomy was performed. Intraoperatively, a massive hemoperitoneum was verified with the presence of a large tumor with a diameter of about 25 cm, which originated from the wall of the urinary bladder and was actively bleeding. After controlling the bleeding, a complete excision of the tumor mass was performed. Pathohistological and immunohistochemical analysis confirmed the diagnosis of extragonadal seminoma. Clinical examination and evaluation of the testes revealed no abnormalities, thus confirming the primary extragonadal localization.

Conclusion Primary extragonadal seminoma of the bladder wall is an extremely rare condition that can manifest with massive hemoperitoneum and hemorrhagic shock. In hemodynamically unstable patients, urgent surgical exploration plays a key role in controlling bleeding and establishing a definitive diagnosis.

Keywords: extragonadal seminoma; germ cell tumor; urinary bladder; hemoperitoneum; hemorrhagic shock

INTRODUCTION

Germ cell tumors are the most common malignant tumors in younger men and in most cases arise in the testicles [1]. However, approximately 2–5% of all germ cell tumors occur in extragonadal locations, most often along the midline of the body, including the mediastinum, retroperitoneum, and central nervous system [2]. The mechanism of their origin has not been fully elucidated, and the most accepted theory implies the lag of primordial germ cells during embryonic migration. Histologically, seminoma represents one of the most common subtypes of extragonadal germinal tumors and is characterized by high sensitivity to therapy based on platinum preparations, which is why timely diagnosis has great prognostic significance [3].

Primary extragonadal seminoma of the urinary bladder is an extremely rare clinical entity. The available literature primarily consists of individual case reports, resulting in a lack of defined diagnostic or therapeutic guidelines. The clinical picture is usually non-specific and includes hematuria, symptoms of the lower urinary tract, or pain in the small pelvis, while the definitive diagnosis is usually made only after the pathohistological and immunohistochemical analysis of the operative preparation. At the same time, it is necessary to rule out the existence of a primary testicular tumor by clinical examination and ultrasound examination of the scrotum in order to confirm the extragonadal nature of the disease [3, 4].

Even rarer is the presentation of extragonadal seminoma as an acute abdomen due to spontaneous intra-abdominal hemorrhage. In such cases, patients usually present with a clinical picture of hemorrhagic shock, which is why urgent surgical intervention is necessary, while the final diagnosis is made only after histopathological and immunohistochemical analysis. Precisely because of the extreme rarity of this clinical presentation, the presentation of individual cases has a significant contribution to a better understanding of diagnostic and therapeutic challenges [3, 5].

In this paper, we present the case of a 36-year-old man with a primary extragonadal seminoma originating from the bladder wall and presenting with massive hemoperitoneum, hemorrhagic shock, and clinical presentation of an acute abdomen, which required urgent exploratory laparotomy and tumor excision.

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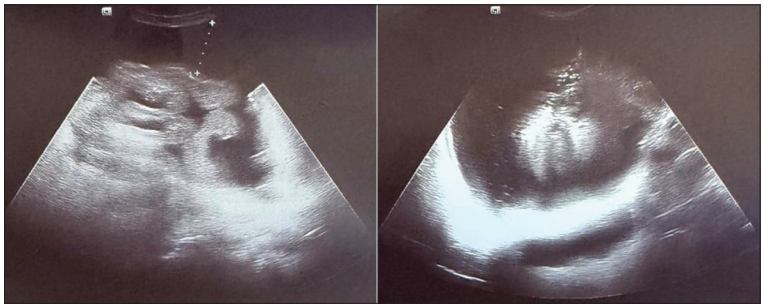


Figure 1. Abdominal ultrasound examination at admission of the patient showing a large amount of free intraperitoneal fluid

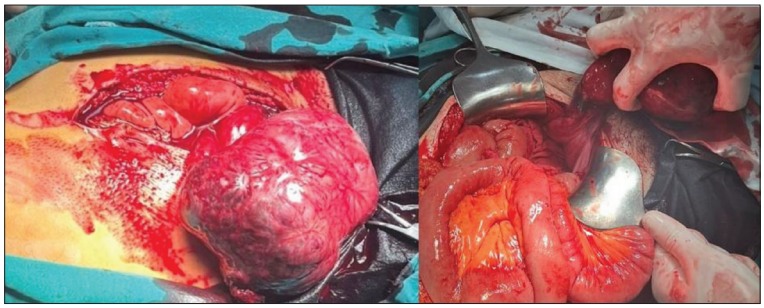


Figure 2. Intraoperative view of a tumor mass originating from the bladder wall with the presence of hemoperitoneum

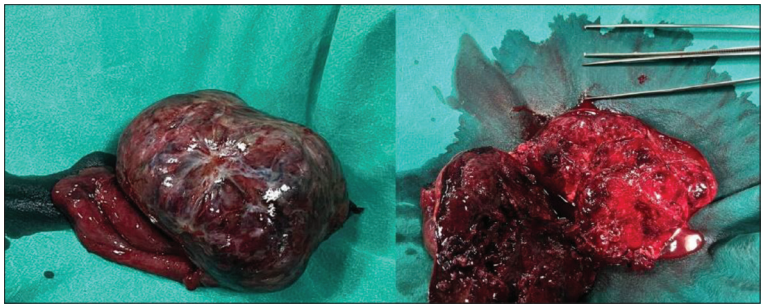


Figure 3. Macroscopic appearance of the tumor after surgical excision: solid tumor mass with hemorrhagic zones and cross-section of the tumor

CASE REPORT

A 36-year-old male patient was admitted to the Surgical Clinic due to the sudden onset of severe abdominal pain, followed by a progressive worsening of the general condition. On physical examination, the abdomen was diffusely tender, with pronounced muscular defense and signs of generalized peritoneal irritation, while the clinical picture of the patient corresponded to hemorrhagic shock, which required immediate resuscitation and urgent surgical intervention.

Initial laboratory tests showed mild leukocytosis with neutrophilia (white blood cells 10.32×10^9/L, neutrophils 83.99%), anemia (red blood cells 2.8×10^{12}/L, hemoglobin 58 g/L), hypoalbuminemia (34 g/L), hypoproteinemia (57.7 g/L), elevated inflammatory markers (C-reactive protein 314.8 mg/L), elevated creatine kinase (347 U/L) and CK-MB (29.3 U/L), while serum lactate was within normal limits (0.7 mmol/L). Serum tumor markers (received postoperatively) showed normal values of alpha-fetoprotein (AFP) (2.99 ng/mL), carcinoembryonic antigen (3.18 ng/mL), carbohydrate antigen 19-9 (19-9, 0.6 U/mL), prostate-specific antigen (PSA, 0.568 ng/mL), and free PSA (0.286 ng/ml), while neuron-specific enolase (NSE) was significantly elevated (148.7 ng/mL).

Abdominal ultrasound examination showed a large amount of free intraperitoneal fluid, interintestinal, intestinal coils dilated and swimming in fluid (Figure 1). A native radiograph of the abdomen showed several air-liquid levels, without signs of pneumoperitoneum.

Considering the patient's hemodynamic instability, diffuse signs of peritoneal irritation and clinical suspicion of intra-abdominal hemorrhage, an urgent exploratory laparotomy was performed without additional radiological processing. Upon entering the abdominal cavity, a massive hemoperitoneum with a large amount of fresh blood and blood clots was found. After the evacuation of the sanguineous contents, a large hemorrhagic tumor with a diameter of about 25 cm was identified by detailed exploration, which originated from the wall of the urinary bladder. The tumor was actively bleeding and was the source of intra-abdominal bleeding responsible for the onset of hemorrhagic shock. Complete surgical excision of the tumor was performed with careful hemostasis, and the specimen was sent for pathohistological and immunohistochemical analysis (Figure 2).

Macroscopic pathological findings showed a large solid hemorrhagic tumor with marked zones of fresh and organized bleeding (Figure 3). Microscopic findings revealed mostly cells with circular, hyperchromatic and partly hypochromatic nuclei with visible nucleoli, basophilic cytoplasm. The entire tumor is permeated with numerous blood vessels, especially subcapsular. Edema of the stroma and microcysts can also be observed in parts. Fields of massive necrosis as well as rare mitoses are present. Immunohistochemical analysis made a definitive diagnosis of extragonadal seminoma (Figure 4). Immunoprofile of the tumor: CD117+, D2-40+, HCG+, OCT3/4+/-, AFP-, CD30-, GATA3-. Ki67 proliferative index is about 30%. Extensive necrosis is present, as is vascular invasion.

Subsequent clinical and ultrasonographic examination of the testicles revealed no palpable abnormalities, which supported the diagnosis of primary extragonadal seminoma and not metastatic disease originating from an occult testicular primary tumor.

The postoperative course went smoothly, and the patient was referred to the oncology board for further staging and planning of multidisciplinary treatment. The oncology board made a decision on systemic chemotherapy according to the PEB regimen (Platinol/cisplatin, etoposide, and bleomycin).

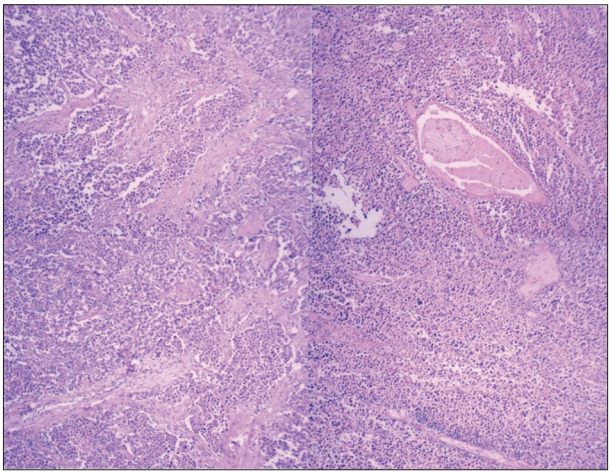


Figure 4. Tumor profile (magnification 10 × 0.25)

DISCUSSION

Extragonadal germ cell tumors represent a rare group of malignancies and account for approximately 2–5% of all germ cell tumors. They most often occur in the mediastinum, retroperitoneum and pineal region, while localization in the urinary bladder is extremely unusual [2]. The pathogenesis of these tumors has not been fully elucidated, and the most accepted theory implies a disturbance in the migration of primordial germ cells during embryogenesis, with their retention in ectopic locations [6]. Another theory supports the existence of an occult or regressed ("burned-out") primary testicular tumor. In our case, the absence of clinical testicular abnormalities was in favor of a primary extragonadal origin of the tumor [3, 4].

The special value of the presented case is the extremely rare location of the tumor. In the available literature, only individual cases of seminoma involving the urinary bladder are described, whereby most of the described cases are the result of secondary spread of the tumor originating from the testicles or other extravesical localizations [7]. Primary extragonadal seminoma of the bladder wall is a clinical entity of which there are only sporadic reports. Therefore, there are no clear recommendations on the optimal diagnostic and therapeutic approach, and most conclusions are based on experiences from individual cases. The tumor in our patient was large (about 25 cm), which is probably the result of slow growth and the absence of specific symptoms in the early stages of the disease [3, 4, 5].

The clinical presentation of our patient was significantly different from the previously described cases. The most common symptoms of extragonadal seminomas involving the urinary bladder include hematuria, dysuria, lower urinary tract obstructive symptoms, or pelvic pain [3]. In contrast, our patient presented with an acute abdomen, hemorrhagic shock and massive hemoperitoneum. This presentation is extremely rare even in intra-abdominal seminomas. Sporadic cases of spontaneous rupture of seminoma in an undescended testis or bleeding of metastatic retroperitoneal masses with the development of hemoperitoneum are described in the literature, but data on primary extragonadal seminoma of the bladder wall leading to intra-abdominal hemorrhage are practically non-existent [8, 9, 10].

In the diagnostic sense, this case indicates the importance of timely surgical decision-making in patients with unclear etiology of hemoperitoneum and hemodynamic instability. The ultrasound finding of a large amount of free fluid and the absence of pneumoperitoneum on the native radiograph did not allow establishing an etiological diagnosis, but, together with the clinical picture of peritoneal irritation and hemorrhagic shock, represented a clear indication for urgent exploratory laparotomy. In such situations, postponing operative treatment for the sake of additional diagnostics can worsen the outcome and endanger the patient's life. In our patient, the definitive diagnosis was made only after intraoperative findings and pathohistological analysis of the preparation [3, 8, 9].

Pathohistological and immunohistochemical analysis play a key role in the diagnosis of extragonadal seminoma. Morphologically, the tumor is characterized by uniform large cells with light cytoplasm and pronounced nucleoli, arranged in nests separated by fibrous septa with lymphocytic infiltrate. Immunohistochemical markers such as PLAP, OCT3/4, SALL4 and CD117 represent an important aid in confirming the diagnosis and differentiating seminoma from other malignant bladder tumors, including poorly differentiated urothelial carcinomas, lymphomas and other "small round cell" tumors [3, 4]. In our case, immunohistochemical analysis was of crucial importance for establishing the final diagnosis.

The therapeutic approach to extragonadal seminomas is based on multimodal treatment that includes surgery, systemic chemotherapy and, in some cases, radiotherapy. However, in patients with acute intra-abdominal hemorrhage and hemorrhagic shock, the priority is urgent surgical control of bleeding and removal of the tumor mass. In our case, the operation had not only therapeutic, but also diagnostic significance, as it enabled a definitive diagnosis and further planning of oncological treatment. Good sensitivity of seminoma to cisplatin-based chemotherapy represents an important prognostic factor and allows a favorable outcome even in advanced forms of the disease [3, 4].

Primary extragonadal seminoma of the urinary bladder is an extremely rare entity that can present atypically, including acute abdomen due to massive intra-abdominal hemorrhage. In hemodynamically unstable patients, urgent surgical exploration can be of crucial importance to control bleeding and establish a definitive diagnosis. Pathohistological and immunohistochemical analysis play a key role in confirming the diagnosis and planning further oncological treatment.

Ethical compliance statement: We confirm that we have read the journal's position on issues involving ethical publication and affirm that work is consistent with those guidelines.

Ethical standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest: None declared.

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Примарни екстрагонадални семином мокраћне бешике са спонтаним хемоперитонеумом и хеморагичним шоком – приказ ретког случаја

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САЖЕТАК

Увод Екстрагонадални семиноми су ретки тумори герминативних ћелија који се најчешће јављају у медијастинуму и ретроперитонеуму. Примарна локализација у мокраћној бешици изузетно је ретка и представља дијагностички изазов. Акутно испољавање у виду интраабдоминалног крварења и хеморагичног шока до сада је веома ретко описано.

Приказ болесника Приказан је случај 36-годишњег мушкарца који је примљен због јаких болова у стомаку, са клиничком сликом акутног абдомена и хеморагичног шока. Лабораторијске анализе показале су анемију (хемоглобин 58 *g/L*) и изражену инфламаторну реакцију (*C*-реактивни протеин 314,8 *mg/L*). Ултразвучним прегледом абдомена утврђена је велика количина слободне интраперитонеалне течности, док је нативна радиографија абдомена показала неколико хидроаеричних нивоа без знакова пнеумоперитонеума. Због хемодинамске нестабилности и знакова перитонеалног надражаја, извршена је хитна експлоративна лапаротомија. Интраоперативно су утврђени масивни хемоперитонеум и велики тумор пречника око 25 *cm*, који је потицао из зида мокраћне бешике и активно крварио. Након контроле крварења извршена је комплетна ексцизија туморске масе. Патохистолошка и имунохистохемијска анализа потврдиле су дијагнозу екстрагонадалног семинома. Клиничким прегледом и евалуацијом тестиса нису откривене абнормалности, чиме је потврђена примарна екстрагонадална локализација.

Закључак Примарни екстрагонадални семином зида мокраћне бешике изузетно је ретко стање које се може испољити масивним хемоперитонеумом и хеморагичним шоком. Код хемодинамски нестабилних болесника хитна хируршка експлорација има кључну улогу у контроли крварења и постављању дефинитивне дијагнозе.

Кључне речи: екстрагонадални семином; тумор герминативних ћелија; мокраћна бешика; хемоперитонеум; хеморагични шок

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Manual instruments for root canal negotiation and glide path preparation – contemporary aspects

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SUMMARY
Root canal negotiation to the full working length and glide path (GP) preparation prior to mechanical root canal instrumentation reduce the risk of procedural errors and contribute significantly to the success of endodontic therapy. The aim of this narrative review was to critically analyze currently available stainless-steel manual instruments used for root canal negotiation and GP preparation, with an emphasis on their design characteristics, mechanical behavior, and clinical applicability. The manuscript describes instruments manufactured from conventional stainless steel and stainless-steel alloys modified by specific heat treatments to improve their mechanical properties. The cross-sectional geometry and tip design of the instruments were analyzed to provide deeper insight into the effect of these parameters on apical patency and overall instrument reliability during clinical use. The potential advantages and limitations of the analyzed instruments are discussed, along with recommendations for their selection in specific clinical situations, depending on the complexity of the root canal system.
Keywords: manual stainless-steel instruments; initial canal negotiation; glide path; endodontics

INTRODUCTION

The introduction of engine-driven nickel-titanium (NiTi) instruments has significantly improved the predictability, efficiency, and overall quality of root canal instrumentation, enabling adequate hermetic obturation, particularly in canal systems with complex anatomy [1, 2]. Since clinical success remains highly technique-sensitive, the improper use of NiTi systems is frequently associated with procedural complications, most notably instrument fracture, especially in narrow, curved, or calcified canals [1, 3]. These complications are primarily attributed to cyclic fatigue, torsional overload, and the combined effects of these factors [4, 5, 6].

Preliminary procedures such as canal negotiation (CN) and glide path (GP) preparation are recommended prior to engine-driven instrumentation in order to reduce the biomechanical stress on NiTi instruments [4–8]. Canal negotiation refers to the initial exploration and identification of the root canal pathway using small-sized manual instruments, with the aim of reaching the desired working length while maintaining the original canal trajectory. Canal patency refers to the maintenance or confirmation of an unobstructed pathway through the apical portion of the canal using a small, flexible instrument, without intentional enlargement beyond the apical foramen. A glide path is defined as a smooth, reproducible, and continuous pathway extending from the canal orifice to the established working length, which facilitates the safe progression of subsequent shaping instruments (Figure 1). The importance of an adequate glide path in reducing torsional stress, cyclic fatigue, and iatrogenic errors has led to the development of numerous mechanical glide path systems [9].

Consequently, stainless-steel (SS) manual instruments have maintained an important role in contemporary endodontic practice due to their simplicity, cost-effectiveness, tactile feedback, and continuous technological improvements in design and manufacturing [10]. Unlike engine-driven instruments, manual files provide enhanced tactile control and sensory perception, particularly in challenging clinical situations such as severe canal curvature, canal calcification, and retreatment cases. Consequently, manual instruments should not be considered obsolete but rather an essential component of a safe and biologically sound instrumentation strategy.

Contemporary manual endodontic instruments are predominantly manufactured from SS, whereas the utilization of carbon steel has markedly declined due to its inferior flexibility and reduced resistance to corrosion [11, 12]. In recent years, manufacturers have introduced modifications to instrument geometry, tip design, surface treatment, and thermal processing, with the aim of improving mechanical

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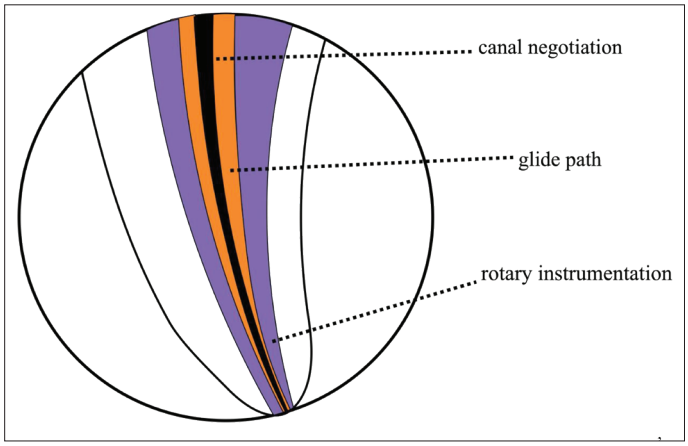


Figure 1. Schematic representation of established initial canal negotiation, prepared glide path, and engine-driven instrumentation

performance and reducing the risk of procedural errors associated with manual instrumentation [8].

These developments have emphasized the need for a critical analysis of contemporary SS manual instruments used for CN and GP preparation, with particular emphasis on how design influences mechanical behavior and clinical applicability, to support informed instrument selection in clinically demanding situations.

METHODOLOGY

This narrative review was based on a literature search conducted in the PubMed, Scopus, and Google Scholar databases using the keywords "manual endodontic instruments," "glide path," "canal negotiation," and "stainless-steel files." The literature search was conducted between January and March 2025. Particular emphasis was placed on peer-reviewed publications addressing the mechanical properties, design characteristics, clinical applications, and performance of manual instruments used for root canal negotiation and glide path preparation. Articles published in English between 1990 and 2025 were included. Additional references were identified through manual searches of relevant journals and reference lists.

Manufacturer-provided information was used primarily to describe instrument-specific technical characteristics, including dimensions, taper, cross-sectional design, material composition, and recommended use, whereas statements concerning mechanical properties, clinical performance, advantages, and limitations were based preferentially on independently published scientific evidence.

TYPES AND CHARACTERISTICS OF MANUAL INSTRUMENTS – OVERVIEW FOR CANAL NEGOTIATION AND GLIDE PATH PREPARATION

Manual instruments remain essential for initial CN and GP preparation, particularly in complex, narrow, or calcified root canals. Their design features, including cross-sectional geometry, tip configuration, and taper, significantly influence mechanical performance, tactile feedback, and clinical safety (Table 1) [13–28].

Critical comparison of manual endodontic instruments – mechanical behavior, handling, and clinical implications

Despite the revolutionary impact of NiTi instruments on contemporary endodontic practice, SS hand instruments remain indispensable, particularly during the critical initial phases of endodontic treatment, when they are used to establish initial canal patency and create the GP. Their enduring clinical relevance stems from their distinct mechanical behavior, which fundamentally differs from that of NiTi alloys and provides both unique advantages and inherent limitations.

From a mechanical perspective, the behavior of manual endodontic instruments is determined not only by the properties of the alloy from which they are manufactured but also by several interrelated design and manufacturing factors, including instrument size, cross-sectional geometry, taper, tip configuration, manufacturing process, and, where applicable, thermal treatment. These variables collectively influence the balance between rigidity, flexibility, buckling resistance, torsional behavior, and tactile feedback [13–28].

From a mechanical perspective, SS instruments exhibit a significantly higher modulus of elasticity (SS ≈ 190–210 GPa, NiTi alloy ≈ 30–50 GPa) and, consequently, greater hardness in comparison with NiTi instruments [12]. This results in increased resistance to torsional stress and buckling under axial load, as well as more pronounced plastic deformation prior to fracture, manifested as visible unwinding of SS instruments before failure [11, 12]. These mechanical properties represent a key advantage during the negotiation of narrow and calcified canals, since the application of apically directed force is often required to overcome anatomical obstacles while preserving tactile control.

However, the increased rigidity of SS instruments represents a double-edged sword. A higher modulus of elasticity implies reduced flexibility, and although enhanced resistance to buckling facilitates apical penetration, excessive stiffness may compromise the instrument's ability to conform to canal curvature. Consequently, the risk of iatrogenic errors, such as canal transportation, ledge formation, or even perforation, may increase during the establishment of initial patency [11, 12]. These opposing effects highlight the importance of achieving a balanced mechanical profile rather than pursuing maximal rigidity at all costs.

Buckling resistance – advantage or potential risk?

Resistance to buckling is often cited as a key determinant of the clinical performance of instruments used to establish initial canal patency; however, its clinical relevance requires careful and critical interpretation [18, 29, 30]. Instruments with insufficient buckling resistance, such as

Table 1. Key characteristics of manual instruments for canal negotiation and glide path preparation, with a focus on design, mechanical properties, and clinical applications

Instrument	Material/ treatment	Cross-section	Taper	Sizes (ISO)	Key features	Clinical advantages	Limitations
K-File Kerr Manufacturing Co. (Romulus, MI, USA)	Stainless steel	Square	2%	06, 08, 10, 15, 20	Twisted design; reliable torsional resistance	Effective in straight/ moderately curved canals; basic CN and GP	Risk of ledging in severe curvature; debris accumulation
K-Reamer Kerr Manufacturing Co. (Romulus, MI, USA)	Stainless steel	Triangular	2%	06, 08, 10, 15, 20	Longer flutes; flexible	Efficient debris removal; flexible for initial GP	Aggressive tip increases risk of zipping/ transportation
C-File Dentsply (Maillefer, OK, USA)	Heat-treated stainless steel	Square	2%	06, 08, 10, 12.5, 15	Enhanced strength	Negotiation of calcified canals	Less flexible than C+ files
C+ Files Dentsply (Maillefer, OK, USA)	Thermally treated stainless steel	Square; pyramidal tip	2–4% regressive	06, 08, 10, 15	High apical control; electropolished surface	Safe negotiation of obliterated canals; good tactile feedback	Requires careful handling in narrow canals
D-Finders Mani (Tochigi, Japan)	Stainless steel	D-shaped	2%	06, 08, 10, 12, 15	Reduced torsional stress	Safe in narrow/ curved canals; enhanced tactile control	Pre-curving recommended in calcified canals
Glide Finders Mani (Tochigi, Japan)	Stainless steel	Square type; rectangular shaft	2–3% regressive	08, 10, 12, 15	Variable cross-section	Effective GP formation; reduced canal transportation	Less suited for extremely tight canals without pre-flaring
Hi-5® Files Integra Miltex (USA)	Stainless steel	Pentagonal	2%	06, 08, 10, 15	Spirally ground flutes; strong	Excellent patency establishment; durable	Limited sizes; careful activation required
Pathfinder CS, SybronEndo (CA, USA)	Carbon steel	Square	2%	K1–07, K2–09	Enhanced cutting strength	Negotiation of tight canals	Corrosion-prone; cannot be autoclaved
S-Finders JS Dental (Sweden)	Stainless steel	Partial circle with flat edges	2%	08	Shorter length facilitates access	Suitable for blocked/obliterated canals	Limited flexibility in severely curved canals
C-PILOT Files VDW (Munich, Germany)	Heat-treated steel	Square	2%	06, 08, 10, 12, 15	Non-cutting pilot tip	Guided progression; reduced ledging	Careful handling needed in narrow canals
Ready-Steel FlexoFiles Dentsply (Maillefer, OK, USA)	Stainless steel	Triangular/ square	2%	06, 08, 10, 12.5, 15, 17, 20	Non-cutting Batt® tip	Safe initial CN and GP; gradual instrumentation	Less efficient in calcified canals
Senseus FlexoFiles Dentsply (Maillefer, OK, USA)	Thermally treated stainless steel	Square	2%	06, 08, 10, 15, 20	Enhanced flexibility	Better tactile feedback; suitable for complex canals	May require use of the balanced force technique

*Table modified from Jovanović-Medojević et al. [9]

small-sized conventional K-files (ISO 06 and 08), are prone to early deformation when encountering apical resistance, limiting their ability to advance in narrow or calcified canals [8]. This deformation, whether in the form of elastic bending or permanent plastic deformation, compromises tactile sensation, reduces cutting efficiency, and increases operator fatigue and frustration.

Conversely, instruments with very high buckling resistance, primarily C+ files, exhibit superior apical penetration capacity and increased resistance to deformation under axial load [29, 30, 31]. Numerous independent studies consistently rank C+ files as having the highest buckling and torsional resistance among conventional K-files, C-PILOT files, and various NiTi GP instruments [32–35]. These mechanical properties make C+ files particularly suitable for negotiating severely narrowed or calcified canals, where the application of apical force is often unavoidable.

However, high buckling resistance should not be interpreted as an unequivocal clinical advantage, as this mechanical property may also represent a clinical risk. The same characteristics that provide increased axial strength can predispose highly rigid instruments to iatrogenic complications. Reduced flexibility limits the instrument's ability to follow canal curvatures, increasing the likelihood of deviation from the original anatomy. Das et al. [30] warned that increased rigidity of C+ files may increase the risk of iatrogenic errors when instruments are used without adequate pre-bending and careful tactile control. Canal transportation studies indicate that excessively rigid instruments, especially those with active cutting tips, can increase the frequency of canal aberrations if used aggressively or without proper pre-bending and tactile guidance [13, 35].

In this context, C-PILOT files have been proposed as a more conservative alternative. By combining thermally

modified SS with a passive, guided tip, these instruments provide a balanced compromise between mechanical strength and preservation of canal anatomy, enabling more predictable negotiation and reducing the risk of procedural errors [29, 33].

The study by Yörel et al. [36], which investigated the preparation of curved and narrow root canals in primary molars, showed that K-files produced less apical transportation than OneShape (MicroMega, Besançon, France) and pediatric rotary EndoArt Pedo Blue files (İnci Dental, Istanbul, Turkey). While the results confirm that the use of rotary instrumentation in primary teeth is safe, K-files provide enhanced tactile sensation and facilitate the formation of an apical matrix, representing an important clinical factor in reducing the risk of material extrusion toward the permanent tooth germs during instrumentation.

Although laboratory measurements of buckling resistance provide valuable information regarding the mechanical behavior of endodontic instruments, these findings should not be interpreted as direct predictors of clinical treatment outcomes.

Influence of manufacturing quality and metallurgy

A critical comparison of manual endodontic instruments must also consider manufacturing quality and metallurgical consistency. Buckling resistance and overall mechanical performance depend not only on instrument design or alloy type but are also significantly influenced by the manufacturing process [30]. Izadi et al. [37] reported a higher incidence of surface defects and irregularities in certain SS instruments, including Mani K-files, which may act as stress concentrators and negatively affect mechanical durability. These findings were corroborated by Nair et al. [38], who demonstrated that Mani K-files exhibited the lowest buckling resistance among the tested pathfinding instruments. These results clearly indicate that not all SS instruments perform equally, and alloy selection alone does not guarantee optimal mechanical behavior.

Thermal treatment of SS has proven to be a decisive factor in improving mechanical properties. Thermally modified SS instruments, such as C-files, C+ files, and C-PILOT files, consistently demonstrate higher buckling resistance compared with conventional SS instruments, and even outperform rotary NiTi GP systems such as PathFile (Dentsply Maillefer, OK, USA) [29–32]. In contrast, carbon steel instruments, such as the Pathfinder CS, offer excellent initial cutting efficiency and high rigidity, but are limited by increased susceptibility to corrosion in the presence of sodium hypochlorite and progressive degradation after repeated sterilization cycles, reducing their long-term clinical reliability [39].

Comparative studies by Kwak et al. [40] and Gupta et al. [18] further confirm these findings, providing strong evidence that advanced, thermally treated SS instruments exhibit significantly greater buckling resistance than NiTi GP instruments. Recent research consistently shows that C+ files display the highest buckling resistance among both manual and engine-driven GP instruments. Although Hamid et al. [41] primarily investigated the metallurgical behavior of thermally treated NiTi instruments, their results further emphasize the importance of alloy processing and phase transformations in determining mechanical properties, highlighting the broader relevance of metallurgical optimization in endodontic instrument design.

Design trade-offs – flexibility *vs*. control

Instrument design plays a key role in modulating the balance between rigidity, flexibility, and tactile perception during manual CN [42]. The instrument's cross-sectional geometry is one of the main determinants of its mechanical behavior. Square and pentagonal cross-sections increase the amount of metal and the core diameter, enhancing buckling resistance and tactile feedback, but at the cost of reduced flexibility [13, 30]. Such designs provide better control in narrow or calcified canals but are less tolerant in curved anatomies.

In contrast, D-shaped and S-shaped cross-sections, characteristic of instruments such as D-Finder and S-Finder, reduce the metal mass and core stiffness, resulting in greater flexibility and improved adaptation to canal curvature. Although these designs may help preserve the original canal anatomy, the reduced core mass is associated with lower buckling resistance. Therefore, these instruments may be less efficient in severely narrowed canals unless used with careful pre-bending, gentle apical pressure, and a controlled manual technique [13, 30].

Tip design represents another crucial variable influencing the clinical behavior of the instrument. Active, pyramidal tips facilitate penetration through calcified obstructions but inherently increase the risk of canal deviation or ledge formation if excessive axial force is applied [22]. Conversely, passive, conical tips offer a safer and more anatomically conservative approach but may be less efficient in penetrating dense calcifications. Das et al. [30] emphasize that although C+ files offer clear advantages in negotiating narrow canals, their increased rigidity and active cutting tip require cautious use to minimize iatrogenic risk. In contrast, C-PILOT instruments have demonstrated more predictable canal tracking due to their guided-tip design, reducing the likelihood of canal aberrations. Thus, instrument selection should not rely solely on canal anatomy but should also consider tactile sensitivity, operator experience, and the ability to precisely control applied force.

Manual *vs*. engine-driven glide path preparation – a complementary relationship

A GP can be prepared using SS hand files or engine-driven NiTi instruments. Although numerous studies have reported potential advantages of engine-driven NiTi GP systems, including reduced instrumentation time, improved centering ability, preservation of the original canal morphology, and decreased extrusion of dentin debris [4, 42–48], their clinical performance may vary according to the specific instrument design, dimensions, alloy characteristics, kinematics, root canal anatomy, and clinical

Table 2. Advantages and disadvantages of manual and engine-driven instruments for glide path preparation

Feature/aspect	Manual stainless-steel instruments	Engine-driven NiTi instruments
Efficiency	Slower preparation; more operator fatigue	Faster preparation (40–50% time reduction); less fatigue
Tactile feedback	Excellent; allows the operator to sense canal irregularities and obstacles	Reduced compared with that of manual instruments; relies on controlled motor settings
Flexibility and canal anatomy preservation	Limited flexibility; risk of canal transportation in narrow or curved canals	Generally greater flexibility; may facilitate preservation of canal morphology depending on instrument design, alloy, dimensions, and clinical conditions
Cutting efficiency	High cutting efficiency in narrow canals; may produce more debris	Efficient cutting; smoother canal shaping; less debris compaction
Mechanical resistance	High torsional and fracture resistance; resistant to corrosion	NiTi instruments are more prone to cyclic fatigue and fracture, especially in complex canals
Debris extrusion / apical irritation	Higher risk of debris extrusion and postoperative pain	May reduce apical debris extrusion in some clinical and laboratory conditions, although outcomes depend on the instrumentation system and technique
Clinical applicability	Essential for initial canal negotiation, especially in calcified or curved canals; cost-effective	Ideal for creating reproducible glide paths; requires investment in equipment and training
Control/safety	High tactile control; allows pre-curving	Dependent on operator skill and motor settings; less tactile feedback
Limitations	Time-consuming; may alter canal shape; risk of procedural errors in complex canals	Risk of instrument fracture; higher cost; learning curve for safe use

situation. Therefore, the choice between manual and engine-driven GP preparation should be individualized rather than based on the assumption that one approach is universally superior.

In many clinical situations, successful manual CN provides an important foundation for subsequent engine-driven GP preparation, particularly in narrow, curved, calcified, or anatomically complex canals. However, the extent of prior manual preparation required may vary depending on the specific NiTi system and the clinical conditions. NiTi GP instruments are generally designed to follow an established canal pathway and may be less suitable for initial exploration of severely calcified or obstructed canals without prior manual negotiation [8, 28]. The advantages and disadvantages of manual and engine-driven instruments for GP preparation are summarized in Table 2.

Although certain instruments, such as the ProTaper Ultimate Slyder (Dentsply Sirona, USA), reportedly achieve initial canal patency in a substantial number of cases (approximately 63%) without prior use of hand instruments, this approach remains a matter of professional debate [19]. In complex canal systems, the prior use of SS hand instruments is considered a safer and more predictable approach for ensuring canal patency compared with applying increased apical pressure to the tip of an engine-driven instrument [4].

Accordingly, manual SS instruments remain the cornerstone of initial CN. Their superior tactile feedback enables the clinician to detect subtle anatomical variations, recognize curvatures or obstructions, and adjust the instrumentation strategy in real time [49]. However, the clinical performance of manual instruments is highly operator-dependent. Technique, experience, and tactile skill play a decisive role in treatment outcomes. This concept is supported by the findings of Allen et al. [13], who observed file deformation in only a subset of operators during simulated use, suggesting that operator-related factors may be as significant as instrument design.

Overall, manual SS and engine-driven NiTi instruments should be regarded as complementary rather than competing approaches to CN and GP preparation. Their appropriate use should be determined by the instrument's specific characteristics and the clinical situation, including root canal anatomy, degree of calcification, canal curvature, and procedural complexity.

CLINICAL IMPLICATIONS

In general, the available evidence indicates that no single manual instrument design represents a universal solution for all clinical situations. Instruments with high buckling resistance are particularly useful for negotiating calcified or severely narrowed canals but require careful use to minimize the risk of iatrogenic damage. Conversely, more flexible designs better preserve canal anatomy in curved canals but may lack the mechanical strength needed to overcome dense obstructions [12].

In addition to instrument-related characteristics, the clinical behavior of manual instruments is strongly influenced by operator-related factors. Operator experience and tactile control are particularly important during CN, while appropriate pre-curving of the instrument may facilitate its adaptation to canal curvature and reduce the risk of deviation from the original canal path. Furthermore, the magnitude and direction of the applied apical force should be carefully controlled, as excessive force may increase the risk of instrument deformation and iatrogenic errors [49].

Therefore, contemporary endodontic practice should favor a selective and sequential approach to instrument use, combining manual SS instruments for initial CN with engine-driven NiTi systems for GP enlargement and further preparation. This staged, hybrid approach reflects

a balance between mechanical efficiency and biological safety, aligning instrument selection with canal anatomy, clinical objectives, instrument characteristics, and operator-related factors, including clinical experience and tactile control [4, 8].

Selection of manual endodontic instruments according to clinical conditions

The selection of appropriate instruments may significantly influence the course and outcome of treatment. During initial CN and GP preparation, the clinical scenario helps determine which instrument may be the safest and most appropriate to use. The manual instruments analyzed in this review possess distinct characteristics that may make them suitable for different clinical conditions. The recommendations presented in Table 3 should be regarded as an evidence-informed clinical framework rather than a rigid protocol and should be adapted individually to root canal anatomy, case complexity, and operator experience.

Table 3. Overview of commonly used or recommended manual instruments for canal negotiation and glide path preparation based on case complexity

Case category	Canal characteristics	Recommended instruments
Simple	Straight canal anatomy, no calcifications	K-Files (Kerr Manufacturing Co.) K-Reamers (Kerr Manufacturing Co.) ReadySteel FlexoFiles (Dentsply)
Moderate	Mild curvature, initial calcification, partially restricted canal patency	Senseus FlexoFiles (Dentsply) Glide Finders (Mani)* D-Finders (Mani) C-PILOT Files (VDW)
Complex	Severe curvature, calcifications, difficult canal negotiation	C+ Files (Dentsply) S-Finders (JS Dental) Hi-5® Files (Integra) Pathfinder CS (SybronEndo) C-Files (Dentsply)

*Instruments primarily designed for glide path preparation

CONCLUSION

The integration of an individualized approach to canal preparation and instrumentation, together with the adoption of new technological solutions, enables successful and predictable endodontic procedures. NiTi systems may offer advantages in terms of flexibility, efficiency, and preservation of canal morphology when an adequate initial canal pathway has been established. Nevertheless, manual instruments remain indispensable during the CN phase. Their adequate mechanical properties, availability, cost-effectiveness, and simplicity of use ensure their continued relevance in contemporary endodontic practice, particularly during the initial stages of treatment in cases involving complex canal morphologies, calcified root canals, or both.

Ethics: The authors declare that the article was written in accordance with the ethical standards of the Serbian Archives of Medicine and the medical institutions with which each author is affiliated.

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Ручни ендодонтски инструменти за успостављање иницијалне проходности и формирање инструментационе путање – савремени приступ

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САЖЕТАК

Успостављање иницијалне проходности канала до пуне радне дужине и формирање инструментационе путање пре машинске инструментације канала корена зуба смањују ризик од настанка процедуралних грешака и значајно доприносе успеху ендодонтске терапије. Циљ овог наративног прегледа био је да се критички анализирају тренутно доступни ручни инструменти од нерђајућег челика који се користе за успостављање иницијалне проходности и припрему инструментационе путање, са посебним нагласком на њихове дизајнерске карактеристике, механичко понашање и клиничку примену. У раду су описани инструменти израђени од конвенционалне легуре нерђајућег челика, као и инструменти чија су својства побољшана применом специфичне термичке обраде ради унапређења њихових механичких карактеристика. Анализирани су попречни пресек и дизајн врха инструмената и утицај ових параметара на поузданост инструмената током њихове примене. Такође су разматране потенцијалне предности и ограничења анализираних инструмената, као и критеријуми за њихов избор у специфичним клиничким ситуацијама, у зависности од сложености система коренских канала.

Кључне речи: ручни инструменти; иницијална проходност; инструментациона путања; ендодонција

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Multidisciplinary early intervention for children with multiple disabilities: a framework for local health centers – a mini-review

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SUMMARY
Multidisciplinary health support for young children with multiple disabilities remains a critical need. This paper aims to analyze the need for a multidisciplinary approach to early intervention in local health centers, where a team of professionals supports children's growth and development. The study employed a desk research approach, a descriptive method, and content analysis. The content of relevant literature was analyzed, and a functional analysis was applied in the research phase of the study. The frameworks for effective multidisciplinary work by different specialists in a local health center include the structures, roles, approaches, and methods that enable specialists to greatly contribute to early intervention for children. Children with multiple disabilities require monitoring and support in a healthcare setting until the age when early intervention shows its effects in all areas of development. A team-based, multidisciplinary approach to working with children with multiple disabilities at an early age is invaluable for children's well-being and health. The roles of different specialists and therapists on the team are significant. All multidisciplinary team members possess the necessary knowledge and competencies and are valuable team members who care for the youngest children. All local health centers should employ various specialists to work in teams treating multiple disorders in young children, ensuring comprehensive and timely early childhood care.
Keywords: children with disabilities; multiple disabilities; early intervention; health center; multidisciplinary approach

INTRODUCTION

An estimated 317 million children and youth were living with health conditions leading to developmental disabilities (such as cerebral palsy, autism, intellectual impairments, and hearing or vision loss) in 2019, highlighting the need for multisectoral systems of support [1]. In Serbia, an estimated 18% of children under age six have developmental disabilities requiring early intervention services, while precise data are still lacking. Early intervention – defined as services provided to children from birth to age three (or 5–6 in some systems) with or at risk of developmental delays – is critical for optimizing developmental outcomes [2].

A multidisciplinary team of experts in a local healthcare facility plays a significant role in early intervention and continuous support for children with multiple disabilities. Despite evidence supporting multidisciplinary early intervention, implementation in local health centers faces significant challenges. These include limited specialist availability, coordination difficulties, inadequate training, funding constraints, and other obstacles. In Serbia, while legislation mandates early intervention services, access varies significantly across regions.

Research shows that a multidisciplinary approach, in which a special therapist works together with a pediatrician, speech therapist, psychologist, physician, and other team members, significantly improves outcomes for children with developmental disabilities (the profession of a special therapist is equivalent to the profession of a special educator and rehabilitator in the Republic of Serbia) [2, 3]. The National Scientific Council on the Developing Child [4] emphasizes that early intervention in the first five years of life is most effective, and an educational psychologist is one of the key professionals who provide adequate stimulation and support. Also, children with multiple disabilities, like children with autism spectrum disorder (ASD), face significantly more difficulties concerning everyday oral hygiene and dental appointments in comparison to their typically developing peers [5], and so dentists who are aware of issues that are specific to this population of patients are important members of the multidisciplinary team. The special therapist participates in multidisciplinary teams for the early assessment of developmental difficulties, using standardized instruments and/or individual assessments for diagnosis and ability assessment [6]. Their role is not limited to the educational context but also encompasses the health, psychological, and social dimensions, as confirmed by both domestic and international research. Within the early development

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counseling services at health centers in Serbia, the special therapist is often the first professional to recognize risks and refer the child for further interventions [7].

This study aims to do the following:

1. To review evidence on effective multidisciplinary team composition and functioning in early intervention;
2. To analyze the roles and competencies of different specialists in supporting children with multiple disabilities;
3. To propose a framework for implementing multidisciplinary early intervention in local health centers.

This study employed a mixed-methods approach combining (1) a systematic literature review and (3) framework analysis of multidisciplinary team models.

We searched the following databases from January 2016 to December 2025: PubMed/MEDLINE, Scopus, Web of Science, ERIC, and Google Scholar. Search terms included combinations of ("early intervention" or "early childhood intervention") and ("multidisciplinary" or "interdisciplinary" or "transdisciplinary") and ("team" or "collaboration") and ("disability" or "developmental delay" or "multiple disabilities") and ("primary care" or "health center" or "community health").

The inclusion criteria were publication in English or Serbian; a focus on children aged 0–6 years; a description of multidisciplinary/interdisciplinary team approaches; and publication as a peer-reviewed article, systematic review, or official guideline.

The exclusion criteria were single-discipline interventions; hospital-based (not community/primary care) settings only; and case reports with fewer than five participants.

In the research, we used a desk research approach, a descriptive method of documentary analysis as described in the literature [8, 9], and content analysis of the literature [8, 10, 11]. The content of the relevant literature was analyzed, and a functional analysis was applied in the research phase of the study to data on children with disabilities who needed multidisciplinary support in the previous period [12].

THE IMPORTANCE OF EARLY IDENTIFICATION AND INTERVENTION FOR CHILDREN WITH DISABILITIES

Early intervention in special education and rehabilitation involves systematic services and therapies for children with developmental risks or disabilities, from birth to ages 5–8. The goal is to enhance the capacity of families to meet the developmental needs of their infants and toddlers, minimize the need for special education and related services when children enter school, and enhance children's long-term outcomes [13]. Research shows that early intervention programs significantly improve the cognitive, social, emotional, and physical development of children with special needs [14].

Globally, over 50 million children under five have mild-to-severe disabilities, predominantly in low- and middle-income countries, with 30 million having moderate-to-severe disabilities [3].

Early intervention reduces the impact of developmental disorders and increases a child's potential for cognitive, social, and psychological growth. Recent reviews show that early rehabilitation in children with global developmental delay has a significant impact on improving outcomes [2]. Other sources confirm that the earlier a child is involved in programs, the more successful it is later on [3], so the optimal timeframe for providing support is by the child's fifth birthday. The brain's greatest neuroplasticity occurs in the first years of life, when it has the greatest ability to change and adapt. The National Scientific Council on the Developing Child [4] notes that in the first few years of life, more than a million new neural connections are formed every second. The American Academy of Pediatrics [15] emphasizes that brain plasticity decreases with age, making early intervention crucial.

Effective early intervention involves a team of specialists: a special therapist, an educational psychologist, a speech therapist, a psychologist, a doctor, and social workers. The family is an active partner – parents receive counseling and support. New models of multidisciplinary support include therapeutic approaches, mediation, and programs for parents [16]. In the United Kingdom, the Supporting Families Programme guidance [17] emphasizes integrated collaboration between institutions and the family.

In Serbia, there is a National Program for the Promotion of Early Childhood Development [18]. Nearly 60,000 children in Serbia are at developmental risk and in need of additional support through early intervention. According to a UNICEF report [19], in Serbia, children with disabilities are significantly less likely to receive early stimulation and responsive care than children without disabilities.

The exact number of children with disabilities in Serbia is not known. The Register of Children with Disabilities, which has been under development by the Batut Institute of Public Health of Serbia since 2021, has provided precise data since 2022 but remains insufficiently developed.

CHILDREN WITH DISABILITIES IN HEALTH CENTERS

Multidisciplinary team members in the health center work with children who may have various developmental and functional disabilities, including motor, intellectual, emotional, sensory, social, behavioral, and learning disorders and disabilities.

Children with motor, developmental, and multiple risks, disorders, and disabilities may have:

1. Disabilities or impairments of the musculoskeletal system, body malformations, and deformities;
2. Neuromuscular diseases and other similar conditions that affect motility and motor skills;
3. Disabilities and impairments of the central and peripheral nervous system (cerebral palsy, epilepsy, hemiplegia, ischemia, brain injury, etc.);
4. Neurodevelopmental disorders, such as ASD, intellectual disability, and attention-deficit/hyperactivity disorder (ADHD);
5. Disabilities and impairments resulting from severe and chronic diseases (cancer, asthma, kidney failure, cardiovascular and endocrine diseases, etc.);

6. Dysfunctions of fine and gross motor skills caused by disorders of premotor integration or other causes;
7. Disharmonious development;
8. Rare diseases with repercussions on the child's motor status;
9. Other disorders, such as feeding and sleep disorders, screen addictions, behavioral disorders, emotional disorders, and learning difficulties [20].

The target group for the work of the multidisciplinary team in the early development counseling services is children from birth to the end of adolescence (18 years of age), particularly at-risk children and children with disabilities. The exact number of children with developmental risks and disorders is not known; only estimates are available.

The authors estimate that more than a billion people live with some form of disability, which is equivalent to approximately 15% of the world's population. Among them, between 110 million (2.2%) and 190 million (3.8%) adults have very significant functional impairments [21].

Assuming that children make up 18% of the population, in Serbia, with a total of about 6,586,476 inhabitants in 2024 [22], there are approximately 1,185,566 children. Of these, about 15% are children with developmental disabilities, or about 177,835 children, and up to 3%, or about 5,335 children, have significant disabilities and functional difficulties of different kinds.

Currently, there are no reliable and representative estimates based on actual measurement of the number of children with disabilities. Existing estimates of childhood disability prevalence vary due to the wide range of methodologies and measurement instruments used. As a result, many children with disabilities may not be identified or receive the necessary services.

Age of children

For work in an early development counseling service, especially in the context of child development, the age of the children can vary depending on the specific needs and services the health center provides. Generally, early development counseling services in local health centers often work with children from early childhood through adolescence.

1. Infants and toddlers (0–3 years):
   – This age is crucial for the development of social, emotional, and cognitive skills. Early intervention services, developmental assessments, and support for both children and parents may be provided in local health centers.
2. Preschool age (3–6 years):
   – Working with preschool-aged children involves assessing development, emotional regulation, and social skills. Additionally, treatments can be provided for the children, and counseling can be offered to parents and educators to ensure the child's optimal development.
3. Elementary school-aged children (6–12 years):
   – At this age, assessments are often conducted regarding academic achievement and skills, emotional health, social skills, communication, and behavior. Children may be involved in therapies or counseling related to anxiety, ADHD, learning disabilities, and other specific challenges. Work also focuses on the family and educators.
4. Adolescence (12–18 years):
   – This period includes challenges related to identity, social relationships, emotional disturbances, and mental health, among others. Developmental counseling often provides support in the form of therapies, teen counseling, and various forms of support during the growing-up process.

MULTIDISCIPLINARY TEAM IN A HEALTH CENTER

The multidisciplinary team of experts at the local health center for children with multiple developmental disabilities consists of various specialists who recognize the importance of teamwork: a pediatrician, a special therapist, a speech therapist, a psychologist, a dentist, and a physiotherapist. Collaboration and coordination with other professionals, families, caregivers, and volunteers involved with the child are essential to implementing a holistic approach to working with children.

Members of a multidisciplinary team work with individuals with complex, multiple disorders and disabilities – for example, with associated motor, sensory, intellectual, social, and/or communication disorders and developmental disabilities.

Analysis of the literature revealed that effective early intervention teams typically include three core members and one or two supplementary specialists, depending on the child's needs (Table 1).

As the results showed, the most commonly included specialists in early intervention multidisciplinary teams were pediatricians, physical therapists, special/occupational therapists, speech therapists, psychologists, and social workers. Table 1 presents the composition of the multidisciplinary team, responsibilities, and competencies of its members, as outlined in the reviewed studies and documents.

The special therapist implements therapeutic work through individualized programs to develop cognitive, communication, and social skills in children with developmental disabilities [14]. Additionally, the special therapist acts as a liaison between the healthcare and education systems, promoting inclusive practices and collaboration with schools and local communities [17] to foster inclusion and cooperation. Special therapists, similar to occupational therapists, have a broad education in medical, social, behavioral, psychological, psychosocial, and related sciences, which equips them with the attitudes, skills, and knowledge to collaborate with people, individually or in groups or communities. Special therapists can work with all people, including those who have an impairment of body structure or function due to a health condition or whose participation is limited or who are socially excluded because of their disability [23].

Table 1. Multidisciplinary team members, responsibilities, and competencies

Specialist	Primary responsibilities	Key competencies
Pediatrician / developmental pediatrician	Medical oversight, diagnosis	Medical diagnosis, developmental screening, developmental monitoring
Physical therapist	Motor development	Gross motor assessment and intervention
Special therapist	Individualized developmental support for specific developmental domains (motor, communication, behavior, daily living skills, fine motor), family guidance	Child development, family-centered practice, multidisciplinary teamwork, individualized planning, adapting interventions to diverse needs
Occupational therapist	Supporting independence in activities of daily living Fine motor skills, bimanual coordination, and spatial orientation therapy	Task analysis and environmental modification, family-centered and routines-based intervention
Speech-language pathologist	Communication, feeding	Language assessment, alternative and augmentative communication
Psychologist	Cognitive and emotional development	Developmental testing, family support
Social worker	Family support, coordination	Case management, resource navigation

The key competencies of a special therapist working with children with multiple disabilities as well as with non-associated disorders and impairments include knowledge of multiple relevant fields, such as special education and rehabilitation, psychology, neurology, psychiatry, neuropsychology, physiology, etc., and various skills necessary for professional teamwork, such as applying a holistic approach, collaboration, developed empathy, and flexibility [24].

A special therapist in a multidisciplinary team conducts a detailed assessment of a child's abilities and determines if the child has motor, sensory, intellectual, social or communication, learning, behavioral, attention, or memory disorders, disabilities, or challenges. In a multidisciplinary team, constant communication among all team members is essential to achieve a comprehensive and coordinated approach in which all participants are actively involved. This is achieved by adapting treatment goals and through continuous monitoring, as well as individual evaluations by each team member for each child. The special therapist collaborates with the other team members and develops an individual rehabilitation plan for the child.

In their work, special therapists use specific standard rehabilitation methods and approaches that stem from the fundamental doctrine of the existence of a biopsychosocial unity within every being.

In addition, special therapists apply current, evidence-based methods, approaches, and rehabilitation programs [25]. Some of the approaches and programs include the following:

- Neurodevelopmental rehabilitation;
- Behavioral methods for working with children with neurodevelopmental disorders;
- Augmentative and alternative communication;
- Application of assistive technology;
- Play therapy;
- Montessori therapy;
- Psychomotor re-education;
- Methods that are not yet evidence-based, such as sensory integration in controlled conditions.

The structure of multidisciplinary team members' work in early development counseling services within a local health center

Team coordination models – There are three primary models that have emerged from the literature:

1. Multidisciplinary model: team members work independently, sharing information periodically.
2. Interdisciplinary model: team members work collaboratively with role overlap.
3. Transdisciplinary model: role release and cross-training with the primary service provider.

Our research focuses on a multidisciplinary health center team in early intervention, addressing children with multiple disabilities, and its conceptual model (Figure 1).

The main areas of work of the members of the multidisciplinary team, in addition to treatment, include the following segments.

1. Therapeutic work and rehabilitation for children with developmental disorders and disabilities, primarily those with multiple disabilities

- Early intervention – active participation in team processes for identifying children at risk of disabilities and those with disabilities at an early age.
- Early intervention – therapeutic work with young children who have been identified as having multiple disabilities of various types, such as ASD, cerebral palsy, muscular dystrophy, Down syndrome, and many others.
- Therapeutic work and rehabilitation for disorders and disabilities in preschool and school-aged children.

The special therapist assesses the child's abilities and needs and creates an implementation plan for an individual program tailored to each child, based on the type and severity of their disabilities. The focus is on developing skills such as mobility, independence in various areas, spatial orientation, social skills, and communication skills (verbal or nonverbal). The plan helps to identify goals and overcome physical, mental, communication, and social challenges. The special therapist will help the child perform expected

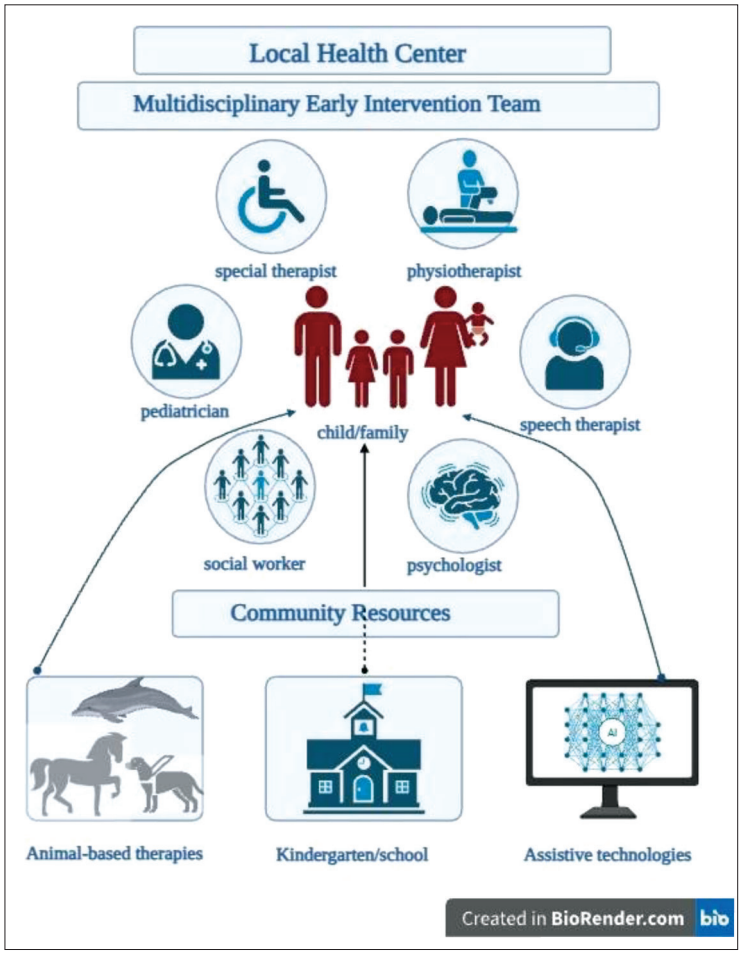


Figure 1. Conceptual model of early-development counseling services

activities in their daily routine and provide the child and family with insight into the resources needed for the child to feel more secure in their environment and more successful in performing various tasks and actions, from different forms of movement (crawling, walking, jumping, running) to actions requiring bimanual movements and coordination (catching, receiving, holding, feeding, etc.) to complex skills and actions with a social and communicative context (graphomotor activities – writing, reading, drawing; counting, speech, memory, attention, memorization, orientation in space and time, etc.).

2. Working with families

Collaboration with a child's family is a significant part of the work of a special therapist and other professionals who work with the child at an early age. Parents of children with disabilities need strong support targeted at strengthening their parental capacities and building special skills, considering their children's disabilities [26]. Experts involve the child's parents (guardians) in the rehabilitation process from the very beginning. The parent is informed about the content and methods of their child's speech and language therapy and is advised to actively participate in the treatment, which is to be continued at home [6]. This can lead to better results in the child's progress in certain areas of work. Parents are also guided on how to encourage their child's overall development, and specifically their advanced skills, through daily home and play activities.

An important area of work is the recognition and prevention of abuse in young children with developmental disabilities or chronic illnesses, who stand out as being more frequently abused at an early age [27].

3. Application of assistive technologies to improve a child's daily functioning

In addition to specialized materials and didactic-play resources, the special therapist also applies appropriate assistive technologies to enable better daily functioning for the child in their activities of daily living. The special therapist trains the child to use the assistive technology and also trains family members. Additionally, other adapted methods, such as alternative and augmentative communication, are introduced and used as needed in the child's activities and daily functioning.

4. Collaboration with other healthcare institutions

The special therapist collaborates with other institutions, such as hospitals, institutes, mental health centers, and various clinics and offices, by performing the following duties:

- Participating in rehabilitation programs in institutions such as day centers and rehabilitation centers.
- Collaborating with pediatricians, physiatrists, neurologists, psychologists, speech therapists, educators, physical therapists, and other specialists.
- Monitoring the development of children with developmental disorders.
- Participating in the preparation and implementation of individualized rehabilitation plans.

5. General prevention and public counseling

The special therapist continuously participates in raising community awareness about the situation and needs of children at risk and those with disabilities and challenges, as well as informing prospective and current parents about the importance of monitoring and encouraging their children's early development. To this end, the special therapist:

- Participates in preventive programs for the early detection of disorders.
- Provides counseling to parents, educators, and medical and other staff.
- Participates in campaigns and the dissemination of useful information.

6. Improving work

In order to continuously improve their work and to monitor and apply modern methods in rehabilitation, the multidisciplinary team members also perform the following tasks and activities:

- Research and analytical work,
- Participation in workshops, seminars, and training sessions,

– Participation in conferences and study trips,
– Keeping up with current literature.

Emerging problematic conditions in children: screen addiction, eating disorders, and sleep disorders

It is no longer a question of whether screen time adversely affects children's early development, but only to what extent.

Data show that multiple developmental and health problems occur in children who overuse all forms of digital media, from digital toys, tablets, phones, computers, and laptops to TV screens [28, 29].

Research shows that children younger than two years old need hands-on exploration and social interaction with parents to properly develop their cognitive, language, motor, and social-emotional skills [30]. Therefore, the use of digital media should be avoided in children younger than 24 months.

Often, due to a lack of awareness, parents do not recognize the signs of screen addiction in their children but only notice individual symptoms without being aware of what caused them [31]. The study by Mićević Karanović et al. [32] confirms that excessive use of digital devices in preschool-aged children can lead to problems with attention, speech, and social interactions.

Eating disorders can occur at an early age in the form of a child's refusal to eat solid food or food of a certain texture, which can be a significant obstacle to the child's proper development and growth. Due to harmful media influence, children can exhibit severe eating disorders like anorexia or bulimia at an unexpectedly early age, but other disorders also occur, such as uncontrollable drinking of water (polydipsia) or eating non-food substances (pica).

Sleep disorders, like eating disorders, can be present on their own or be part of the clinical picture of more complex conditions or disorders. According to the World Health Organization, there is a direct link between screen time for children under five years old and sleep disturbances [29]. Therefore, sleep problems are very significant and need to be addressed at the earliest age.

The preceding findings show that local health centers should have several different specialists for multiple disabilities in their multidisciplinary teams (a pediatrician, a special therapist, a speech therapist, a dentist, a psychologist, and a physiotherapist) to ensure that all children at an early age are provided with adequate and timely health care and protection in the local community, regardless of whether they live in urban or rural areas [33].

CONCLUSION

The multidisciplinary team of different experts who work with young children with multiple disabilities in local health centers plays a significant role in children's development and their health protection. Support services from various specialists, provided promptly as part of early intervention, are extremely beneficial and have a positive impact on the well-being and further development of children with developmental disabilities and challenges in multiple areas. Multidisciplinary support must be provided to all children within early intervention in primary health care in local health centers, regardless of whether they live in urban or rural areas.

Ethics: The authors declare that the article was written in accordance with the ethical standards of Serbian Archives of Medicine and the ethical standards of each author's medical institutions.

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Мултидисциплинарна рана интервенција код деце са вишеструким сметњама: оквир за локалне домове здравља – кратки преглед

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САЖЕТАК

Мултидисциплинарна здравствена подршка деци са вишеструким сметњама у развоју у раном узрасту представља неопходну потребу. Циљ овог рада је да анализира потребу за мултидисциплинарним приступом раној интервенцији у локалним здравственим центрима, где тим стручњака подржава раст и развој деце. Студија је користила приступ секундарног истраживања, дескриптивну методу и анализу садржаја. Анализиран је садржај релевантне литературе, а функционална анализа је примењена у истраживачкој фази студије. Оквири за ефикасан мултидисциплинарни рад различитих специјалиста у локалном здравственом центру укључују структуру, улоге, приступе и методе које их чине важним фактором у раду са децом у области ране интервенције. Деци са вишеструким сметњама у развоју потребни су праћење и подршка у здравственом окружењу до узраста када рана интервенција показује своје ефекте у свим областима развоја. Тимски, мултидисциплинарни приступ и рад са децом са вишеструким сметњама у развоју у раном узрасту непроцењиви су за добробит и здравље деце. Улоге различитих специјалиста и терапеута у тиму су значајне. Сви чланови мултидисциплинарног тима поседују потребна знања и компетенције и значајно доприносе бризи о најмлађој деци. Сви локални здравствени центри требало би да запошљавају различите специјалисте ангажоване у тимовима за третман вишеструких сметњи код деце раног узраста како би се осигурала потпуна и благовремена заштита у раном детињству.

Кључне речи: деца са сметњама у развоју; вишеструке сметње у развоју; рана интервенција; дом здравља; мултидисциплинарни приступ

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Lipids in the diet of children and adolescents

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SUMMARY
Lipids are macronutrients with multiple roles in adequate growth and development, as well as in maintaining health in other phases of life. In addition to being, along with carbohydrates, the main source of calories, lipids are indispensable structural and functional constituents of the human body. Their intake must be complete and adjusted to optimal needs, which differ in quantitative terms during different phases of growth and development. Of particular importance are meeting the needs for essential and semi-essential fatty acids and strictly adhering to the upper limits of permissible intake of saturated fatty acids, cholesterol, and trans fatty acids.
Keywords: lipids; physiological role; optimal needs; children and adolescents

INTRODUCTION

Lipids, along with proteins and carbohydrates, are among the macronutrients of the human diet. In addition to being an important source and reservoir of energy, lipids are essential structural and functional factors of the human body and, as such, are responsible for numerous physiological processes [1–5]. Inadequate fat balance during the developmental period has numerous consequences, such as energy deprivation and essential fatty acid deficiency in celiac disease, cystic fibrosis, and cholestatic syndrome, or obesity, secondary hyperlipidemia, and a tendency to cholelithiasis in conditions of excessive intake [6–13].

This article, along with a review of the dietary content, digestion, absorption, and basic physiological roles of lipids, provides current guidelines for their optimal intake in childhood and adolescence.

LIPIDS IN THE HUMAN DIET

About 90–95% of the lipids present in the standard human diet are triglycerides, with the remainder being phospholipids and cholesterol [1]. Triglycerides are esters of glycerol and fatty acids. According to chain length, fatty acids are classified as short-chain (≤ C6), medium-chain (C8–14), or long-chain (≥ C16); they may also be classified according to whether they contain an even or odd number of carbon atoms [1, 14]. The human diet is dominated by long-chain fatty acids with an even number of carbon atoms. Long-chain fatty acids are further differentiated into saturated and mono- and polyunsaturated. The monounsaturated fatty acids palmitoleic (C16:1, ω-7) and oleic (C18:1, ω-9) can be synthesized in the human body, while the essential fatty acids linoleic (C18:2, ω-6) and alpha-linolenic (C18:3, ω-3) must be ingested through food [15]. The main sources of essential fatty acids are foods of plant origin, because plants, unlike animals, possess Δ12-desaturase in their chloroplasts, which converts oleic acid into linoleic acid, as well as Δ15-desaturase, which transforms linoleic acid into alpha-linolenic acid [16, 17]. Although plant foods are, in general, low in fat, there are certain plant varieties from which oils rich in linoleic and alpha-linolenic acids, as well as other unsaturated fatty acids, are obtained by appropriate technological procedures. During the process of successive elongation and desaturation of essential fatty acids, which is almost completely carried out in the liver, long-chain polyunsaturated fatty acids (LCPUFA) are formed, which have ≥ 20 carbon atoms and ≥ 2 double bonds [1]. However, the capacity for endogenous synthesis of the final components of ω-3 LCPUFA, i.e., docosahexaenoic acid (DHA, C22:6, ω-3) and eicosapentaenoic acid (EPA, C20:5, ω-3), is insufficient not only in infants but also at other ages, so the intake of preformed EPA and DHA with food or in the form of supplements is necessary to meet physiological needs [15, 18–22]. This also applies to arachidonic acid (C20:4, ω-6), which is also a semi-essential nutrient within the first few months after birth [23]. According to the spatial arrangement of hydrogen atoms around the double bond of the carbon chain, cis and trans unsaturated fatty acids are further distinguished. Trans fatty acids, identically to

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saturated ones, are characterized by a rigid linear structure, a high melting point, and high atherogenic potential [24–27]. In addition, some studies suggest that excessive intake of trans fatty acids (> 1% of total energy intake) is a risk factor for developing insulin resistance and breast, prostate, and colon cancers [24, 25]. In milk, dairy products, and ruminant meat, trans fatty acids make up 4–6% of all fatty acids [28]. However, during the thermal processing of food, and especially during the hydrogenation of vegetable oils into margarine, a large part of cis fatty acids, sometimes as much as 60%, is converted to trans isomers [28]. Due to transplacental and transmammary transfer and possible damage to the developing brain, intake of trans fatty acids should be minimized among pregnant and breastfeeding women, as well as children, especially during the first 2–3 years of life [24, 28].

Phospholipids are complexes of diacylglycerol and inositol, choline, serine, or ethanolamine linked by a phosphate group. The exception is sphingomyelin, which contains sphingosine instead of glycerol. As key components of the cell and subcellular membranes of all eukaryotes, phospholipids are present in foods of plant and animal origin.

Cholesterol is the main animal sterol, while the main sterol in fungi is ergosterol, and in plants sitosterol. Cholesterol is synthesized in the body from acetyl-CoA, primarily in the liver, and is partly ingested with animal food. Dietary cholesterol is primarily (85–90%) absorbed in free form and, to a lesser extent, as esters with fatty acids.

Tables 1–3 show the quantitative and qualitative fat content of some foods [29–34].

Plant foods are characterized by the absence of cholesterol, with the exception of palm and coconut oil, the predominant content of unsaturated fatty acids and, except for algal oil, a complete lack of LCPUFA. Also, the level of EPA and DHA in animal food from land is much lower than in food of the same species from water, especially marine [35].

Table 1. Triglyceride and cholesterol content in certain types of food [29, 30]

Type of food	Triglycerides (g/100 g)	Ratio of saturated to unsaturated fatty acids	Cholesterol (mg/100 g)
Human milk*	3.5–4.5	0.34–0.47:1	9–15
Cow's milk	3.6–3.88	1.7–2.3:1	5–15
Cheese (full-fat)	23–32	1.8–2.1:1	70–100
Chicken egg yolks	31–33	1.8–1.1:1	1070–1700
Chicken liver	4–6.2	0.93:1	429–555
Chicken meat (with skin)	6.2–11.2	0.55–0.62:1	60–91
Beef (brisket)	1.55–3	2.32:1	70
Pork (lean)	1–2.8	0.34:1	65–77
Sardine	1.2–9.81	0.43:1	142
Salmon	12.5–16.5	0.28:1	35–52
Catfish	1.58–4.3	0.1:1	33

*According to reference [29]

Table 2. Linoleic and α-linolenic acid content in vegetable and animal fats (mg/100 g) [31, 32]

Dietary source	Linoleic acid	α-linolenic acid	Ratio
Linseed oil	24,000	47,000	1:2
Soybean oil	53,400	7600	7:1
Olive oil	8000	950	8:1
Corn oil	50,000	900	55:1
Sunflower oil	60,200	500	120:1
Pork fat	8600	1000	8.6:1
Tallow	2580	190	13.6:1

Table 3. Content of basic ω-3 long-chain polyunsaturated fatty acids in food (mg/100 g) [33, 34]

Dietary source	EPA	DHA
Sardine	763	1292
Herring	909	1105
Salmon	862	1104
Tuna	91	328
Catfish	20	69
Fish oil	27,050	900
Microalgae oil	15,000	30,000
Human milk	0.47–0.53	3.51–5.52
Infant formula	0.45–0.55	6.81–6.88
Egg	0	58
Chicken	10	20
Beef	2	3
Pork	0	2

DHA – docosahexaenoic acid; EPA – eicosapentaenoic acid

DIGESTION AND ABSORPTION OF LIPIDS

Digestion and absorption of fat occur primarily in the small intestine. Triglycerides are hydrolyzed by pancreatic lipase, phospholipids by phospholipase 2, and cholesterol esters by cholesterol esterase. Under the action of sublingual and gastric lipase, most of the short- and medium-chain triglycerides are hydrolyzed and absorbed in the stomach [36, 37, 38]. In breastfed infants, milk lipase also makes an important contribution to the digestion of triglycerides [36]. After hydrolysis, fatty acids and cholesterol are absorbed and transported into the bloodstream via the lymphatic system as part of chylomicrons [37, 38]. In addition to pancreatic lipase, colipase, and an optimal pH, an optimal amount of bile acids and salts is required for normal digestion and absorption of fats [37, 38]. The exceptions are short-chain and medium-chain triglycerides, which are water-soluble and, independent of bile acids and salts, either in hydrolyzed or intact form, pass directly into the bloodstream [36, 38].

PHYSIOLOGICAL ROLE OF LIPIDS

Lipids are essential structural and functional factors of the human body. Triglycerides, along with carbohydrates, are the main source of energy. In addition, they are the main reservoir of energy and indispensable carriers of numerous physiological functions. The role of phospholipids and cholesterol lies in their universal presence in the cellular and subcellular membranes of all eukaryotes [39, 40]. Cholesterol is a precursor of steroid hormones and bile acids and a basic component of myelin [41, 42]. Essential fatty acids have multiple important roles. Unlike other fatty acids, their primary role is related to the synthesis of

Table 4. Basic guidelines regarding fat intake in the diet of children and adolescents [45, 46, 47]

Component	Recommended intake
Total fat (share of total caloric value of food)	0–1 year: 40–50% 2–3 years: 30–35% 4–18 years: 25–35%
Essential fatty acids	7–10% (ω-6:ω-3 ~ 5–10:1)
Saturated fatty acids	< 10%
Trans fatty acids	< 1%
Cholesterol	< 300 mg daily

Table 5. Recommended daily intake of ω-3 long-chain polyunsaturated fatty acids in food (WHO/FAO, 2008) [49]

Age	DHA	EPA + DHA
0–6 months	0.1–0.18% of total energy intake	
6–24 months	10–12 mg/kg	
2–4 years		100–150 mg
4–6 years		150–200 mg
> 6 years		200–250 mg

DHA – docosahexaenoic acid; EPA – eicosapentaenoic acid

LCPUFA, mostly DHA and arachidonic acid (C20:4, ω-6), which are part of the membrane phospholipids of cells of the central nervous system and retina [15, 20, 23]. When present in adequate amounts, with the highest concentrations in the neurocytes of the gray matter of the frontal lobe of the brain, photoreceptor cells of the macula, and interneural synapses, these LCPUFA fractions, especially DHA, ensure optimal mental and visual function [22, 23, 43]. In addition, LCPUFA are precursors of tissue hormones (eicosanoids), prostanoids, leukotrienes, lipoxins, and others, which play an irreplaceable role in numerous processes at the cellular and tissue levels [22, 23]. DHA is also a precursor of the anti-inflammatory neuroprotectors, protectins, resolvins, and maresins [20, 44].

PHYSIOLOGICAL NEED FOR LIPIDS

Recommendations from the Institute of Medicine of the United States, the American Heart Association, and the United States Department of Health and Human Services regarding the proportion of fat in the diet are given in Table 4 [45, 46, 47]. Both the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommend that saturated fatty acids and trans fatty acids account for less than 10% and less than 1% of total energy intake, respectively [47, 48, 49]. Considering the limited capacity for endogenous conversion of alpha-linolenic acid into EPA and DHA, recommendations are given regarding their optimal intake (Table 5) [49]. Identical or similar recommendations are followed by other relevant international and national institutions and associations [15, 50].

CONCLUSION

Lipids are among the three basic components of the human diet. They are an important source of energy and essential structural and functional constituents of the human body. Their intake must be adapted to optimal needs, which differ quantitatively during different stages of growth and development. Of particular importance are meeting the needs for essential and semi-essential fatty acids and strictly adhering to the permitted intake limits of saturated fatty acids, cholesterol, and trans fatty acids.

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Липиди у исхрани деце и адолесцената

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САЖЕТАК

Липиди су макронутријенти од вишеструког значаја за адекватан раст и развој, као и очување здравља у осталим фазама живота. Сем што су, уз угљене хидрате, основни извор калорија, липиди су неизоставни структурни и функционални конституенти организма. Њихов унос мора бити комплетан и прилагођен оптималним потребама, које се у квантитативном погледу разликују током различитих фаза раста и развоја. Посебан значај има задовољавање потреба за есенцијалним и семиесенцијалним масним киселинама, као и стриктно поштовање горње границе дозвољеног уноса засићених масних киселина, холестерола и трансмасних киселина.

Кључне речи: липиди; физиолошка улога; оптималне потребе; деца и адолесценти

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LETTER TO THE EDITOR / ПИСМО УРЕДНИКУ

Three hours and four deaths – years of life lost as a measure of the burden of motorcyclist fatalities

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SUMMARY
On September 6, 2026, four motorcyclists aged 17, 30, 31, and 46 years died in three traffic crashes in Serbia within a three-hour period. Using 75 years as the upper reference age, these deaths represented 176 potential years of life lost. Assuming a potential working life from 18 to 65 years, they also represented 135 potential working years lost. Valued illustratively at the average monthly gross salary for June 2026, the undiscounted value of lost future gross earnings was approximately RSD 269 million (approximately EUR 2.3 million). This excludes healthcare, emergency response, police and judicial costs, property damage, disability, unpaid work, and intangible family losses. Death counts alone therefore understate the public health and societal burden. Reporting potential years of life lost alongside conventional mortality indicators could support more proportionate prevention policies for motorcyclists and moped users.
Keywords: premature mortality; wounds and injuries; economic burden; accident prevention

Dear Editor,

On September 7, 2026, the Ministry of the Interior of the Republic of Serbia reported that four motorcyclists had died in traffic crashes during a period of only three hours on the previous day [1]. At 17:15, two motorcyclists aged 31 and 46 years died at the scene following a head-on collision between two motorcycles in the Ljig area; a 33-year-old passenger on one of the motorcycles sustained life-threatening injuries. Twenty minutes later, in the municipality of Temerin, a 17-year-old motorcyclist died after losing control of his motorcycle and striking a concrete post. According to the Ministry, he did not have a driver's license and was not wearing a protective helmet or other protective equipment. At 20:15, a 30-year-old motorcyclist died following a collision with a car in the Sremska Mitrovica area [1].

A count of deaths alone does not fully represent the health and societal burden of these events. Potential years of life lost give greater weight to deaths at younger ages and constitute a measure of premature mortality [2]. Using a simple model with an upper reference age of 75 years, the individual losses were 44, 29, 58, and 45 years. Thus, within a single afternoon, these four deaths accounted for a total of 176 potential years of life lost.

The economic consequence may also be expressed approximately as potential working years lost. Assuming, for operational purposes, a working life from 18 to 65 years, the individual losses were 34, 19, 47 and 35 years, amounting to 135 potential working years. If each year is valued, solely as a simplified human-capital indicator, using the average monthly gross salary for June 2026 of RSD 166,123 [approximately EUR 1,420 (converted using an exchange rate of approximately RSD 117.2 per EUR)] [3], the undiscounted value of lost future gross earnings is approximately:

135 × 12 × RSD 166,123 = RSD 269,119,260.

This amount, approximately RSD 269 million (approximately EUR 2.3 million), is not an estimate of the total economic cost of the crashes. It is only an illustrative estimate of lost gross earnings. It does not account for the probability of employment, future wage and productivity growth, discounting, unpaid work, emergency medical and hospital costs, police and judicial expenditure, property damage, long-term disability, or the intangible losses borne by families. The actual societal burden is therefore undoubtedly greater.

The event is even more alarming in light of the Ministry's report that, since the beginning of 2026, traffic crashes have killed 48 motorcyclists and three motorcycle passengers, as well as seven moped riders and two moped passengers. During the corresponding period of the previous year, 39 motorcyclists and one moped rider had died, with no deaths among motorcycle or moped passengers [1]. These figures indicate that deaths among users of powered two-wheelers should be treated as a priority public health problem, consistent with the Safe system approach, which acknowledges that people make mistakes but seeks to prevent those mistakes from resulting in death or serious injury [4].

Prevention efforts must simultaneously address speed control, the use of helmets that meet applicable safety standards and

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other protective equipment, possession of the appropriate driver's license, safer road infrastructure, education of young riders, and the responsibility of those who make vehicles available to minors. Consistent enforcement of laws prohibiting the operation of motorcycles, mopeds, and other motor vehicles under the influence of alcohol or psychoactive substances is also required. This is stated as a general preventive measure and does not imply that any rider killed in the crashes described above was under the influence of alcohol or psychoactive substances, as the Ministry's statement contains no such information.

Four deaths in three hours are not merely four units in mortality statistics. They represent 176 years of life lost, at least 135 years of potential working contribution, and an economic loss that, even under a highly restrictive approach, amounts to hundreds of millions of dinars. Routine reporting of potential years of life lost alongside conventional road traffic mortality indicators could improve recognition of the burden of injury and help direct preventive measures more effectively.

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Три часа и четири смртна исхода – изгубљене године живота као мера терета страдања мотоциклиста

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САЖЕТАК

Дана 6. септембра 2026. године, у три саобраћајне незгоде у Србији, у размаку од три часа, погинула су четири возача мотоцикла старости 17, 30, 31 и 46 година. Уз горњу референтну границу од 75 година, ови смртни исходи представљају 176 потенцијално изгубљених година живота. Ако се потенцијални радни век одреди од 18. до 65. године, изгубљено је и 135 потенцијалних година радног века. Када се оне, искључиво илустративно, вреднују просечном месечном бруто зарадом из јуна 2026. године, недисконтована вредност изгубљених будућих бруто зарада износи приближно 269 милиона динара (око 2,3 милиона евра). Процена не обухвата трошкове здравственог збрињавања, хитних служби, полицијских и судских поступака, материјалну штету, инвалидност, неплаћени рад и нематеријалне губитке породица. Овај прорачун показује да сам број погинулих потцењује јавноздравствени и друштвени терет саобраћајних повреда. Приказивање потенцијално изгубљених година живота уз уобичајене показатеље морталитета могло би допринети примеренијем планирању превенције страдања корисника мотоцикала и мопеда.

Кључне речи: превремена смртност; повреде; економски терет; спречавање незгода

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 позиву, 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 и на који начин рад 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 у довољној мери учествовао 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 речју. 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 – не сме имати више од 5000 речи за оригинални рад, рад из историје медицине и преглед литературе, односно више од 3000 речи за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак, рад за рубрику „Језик медицине“ и извештај са конгреса и научних скупова; 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 CA 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 посебно доставити 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*, словима величине 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *https://www.nlm.*

nih.gov/bsd/uniform_requirements.html. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs/en/submission-letter/SubmissionLetterForm2023.pdf*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА И НАКНАДЕ ЗА ОБРАДУ И ОБЈАВЉИВАЊЕ ЧЛАНКА. Да би рад био разматран за објављивање у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 9 Статута Друштва) у години у којој рад предају на разматрање.

Следеће накнаде су обавезне како би рад био прегледан, обрађен и потенцијално објављен у *Српском архиву за целокупно лекарство*:

- накнада за преглед сваког примљеног рада домаћих аутора: 6.000 динара по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада домаћих аутора: 12.000 динара по раду;
- накнада за преглед сваког примљеног рада страних аутора: 75 евра (или 9000 динара) по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада страних аутора: 150 евра (или 18000 динара) по раду.

Накнаде се плаћају пре прегледања, односно пре објављивања рада. Радови за које нису плаћене накнаде неће бити прегледани, односно објављени.

Треба напоменути да уплата накнаде за преглед рада није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и накнаду за преглед чланка, као доказ о уплатама. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за преглед чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Онлајн систем за подношење радова водиће вас кроз поступак уноса података о чланку и отпремања ваших датотека. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и бројеве телефона.

АДРЕСА:
Српско лекарско друштво
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Before submitting their paper to the Editorial Office of the Serbian Archives of Medicine, authors should read the Instructions for Authors, where they will find all the necessary information on writing their manuscript in accordance with the journal's standards. It is essential that authors prepare their manuscript according to established specifications, as failure to do so will result in the paper being delayed or rejected. Serbian Archives of Medicine provides no fee for published articles. By submitting a paper for publishing consideration, authors of a paper accepted for publication in the Serbian Archives of Medicine grant and assign all copyrights to the publisher – the Serbian Medical Society.

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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 square brackets (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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CONFLICT OF INTEREST STATEMENT. The manuscript must be accompanied by a disclosure statement from all authors (contained within the Submission Letter) declaring any potential interest or stating that the authors have no conflict of interest. For additional information on different types of conflict of interest, please see World Association of Medical Editors (WAME, *www.wame.org*) policy statement on conflict of interest.

AUTHORSHIP. All individuals listed as authors should be qualified for authorship. Every author should have participated sufficiently in writing the article in order to take responsibility for the whole article and results presented in the text. Authorship is based only on: crucial contribution to the article conception, obtaining of results or analysis and interpretation of results; design of manuscript or its critical review of significant intellectual value; final revision of the manuscript being prepared for publication.

The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019, all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with proven plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Archives 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.

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TRANSLATION INTO SERBIAN. The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine," etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

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ABBREVIATIONS. To be used only if appropriate, for very long names of chemical compounds, or as well-known abbreviations (standard abbreviations such as DNA, AIDS, HIV, ATP, etc.). Full meaning of each abbreviation should be indicated when it is first mentioned in the text unless it is a standard unit of measure. No abbreviations are allowed in the title. Abbreviations in the summary should be avoided, but if they have to be used, each of them should be explained when first mentioned in the text of the paper.

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LENGTH OF PAPER. The entire text of the manuscript – title page, summary, the whole text, list of references, all enclosures including captions and legends (tables, photographs, graphs, schemes, sketches), title page and summary in Serbian – must not exceed 5,000 words for original articles, review articles and articles on history of medicine, and 3,000 words for case reports, preliminary and short communications, current topics, articles for practitioners, educational articles, articles for "Language of medicine" and congress and scientific meeting reports; for any other section maximum is 1,500 words.

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ARTICLE ENCLOSURES are tables, figures (photographs, schemes, sketches, graphs) and video-enclosures.

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If the manuscript is entirely in the Serbian language, tables and corresponding legend should be both in Serbian and English. Also, the table cells should contain text in both languages (do not create two separate tables with a single language!).

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Photographs may be printed and published in color, but possible additional expenses are to be covered by the authors.

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ACKNOWLEDGMENT. List all those individuals having contributed to preparation of the article but having not met the criteria of authorship, such as individuals providing technical assistance, assistance in writing the paper or running the department securing general support. Financial aid and all other support in the form of sponsorship, grants, donations of equipment and medications, etc., should be mentioned as well.

REFERENCES. The reference list is the responsibility of the authors. Cited articles should be readily accessible to the journal's readers. Therefore, following each reference, its DOI number and PMID number (if the article is indexed for MEDLINE/PubMed) should be typed. References should be numbered in Arabic numerals in order of citation in the text. The overall number of references should not exceed 30, except in review articles, where maximum of 50 is acceptable, and in meta-analyses, where up to 100 references are allowed. The number of citations of original articles must be at least 80% of the total number of references, and the number of citations of books, chapters and literature reviews less than 20%. If monographs and articles written by Serbian authors could be included in the reference list, the authors are obliged to cite them. The majority of the cited articles should not be older than five years. Use of abstracts as references is not allowed. If it is important to comment on results published solely in the form of an abstract, it is necessary to do so within the text of the article. The references of articles accepted for publication should be designated as *in press* with the enclosed proof of approval for publication.

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